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(71) Applicant: IMMUNEX CORPORATION [US/US]; Law Dept., 51 University Street, Seattle, WA 98101 (US).

(72) Inventors: WALCZAK, Henning; Alte Berheimer Strasse 12, D–69115 Heidelberg (DE). SMITH, Craig, A.; 2405 5th West, Seattle, WA 98119 (US).

(74) Agent: ANDERSON, Kathryn, A.; Immunex Corporation, Law Dept., 51 University Street, Seattle, WA 98101 (US).

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(54) Title: PROTEIN THAT BINDS TRAIL

(57) Abstract

A protein designated TRAIL–BP binds the protein known as TNF–Related Apoptosis–Inducing Ligand (TRAIL). The TRAIL–BP finds use in purifying TRAIL or inhibiting activities thereof. Isolated DNA sequences encoding TRAIL–BP are provided, along with expression vectors containing the DNA sequences, and host cells transformed with such recombinant expression vectors. Antibodies that are immunoreactive with TRAIL–BP are also provided.

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TITLE
PROTEIN THAT BINDS TRAIL

BACKGROUND OF THE INVENTION

A protein known as TNF-related apoptosis-inducing ligand (TRAIL) is a member of the tumor necrosis factor family of ligands (Wiley et al., *Immunity*, 3:673-682, 1995). TRAIL has demonstrated the ability to induce apoptosis of certain transformed cells, including a number of different types of cancer cells as well as virally infected cells (PCT application WO 97/01633 and Wiley et al., *supra*). Identification of cell surface protein(s) that bind TRAIL would prove useful in further elucidating the biological activities of TRAIL.

SUMMARY OF THE INVENTION

The present invention is directed to a novel protein that binds to the protein known as TNF-related apoptosis-inducing ligand (TRAIL), and thus is designated a TRAIL-Binding Protein (TRAIL-BP). DNA encoding TRAIL-BP, and expression vectors comprising such DNA, are provided. A method for producing TRAIL-BP polypeptides comprises culturing host cells transformed with a recombinant expression vector encoding TRAIL-BP, under conditions that promote expression of TRAIL-BP, then recovering the expressed TRAIL-BP polypeptides from the culture. Antibodies that are immunoreactive with TRAIL-BP are also provided.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1 presents the nucleotide sequence of a DNA encoding a human TRAIL-Binding Protein, as well as the amino acid sequence encoded thereby.

DETAILED DESCRIPTION OF THE INVENTION

A novel protein designated TRAIL-Binding Protein (TRAIL-BP) is provided herein. TRAIL-BP binds to the cytokine designated TNF-related apoptosis-inducing ligand (TRAIL). Certain uses of TRAIL-BP flow from this ability to bind TRAIL, as discussed further below. TRAIL-BP finds use in inhibiting biological activities of TRAIL, or in purifying TRAIL by affinity chromatography, for example.

TRAIL-BP protein or immunogenic fragments thereof may be employed as immunogens to generate antibodies that are immunoreactive therewith. In one embodiment of the invention, the antibodies are monoclonal antibodies.

The nucleotide sequence of a human TRAIL-BP cDNA is presented in Figure 1 (SEQ ID NO:1), along with the amino acid sequence encoded by the cDNA (SEQ ID NO:2). The TRAIL-BP protein of Figure 1 (SEQ ID NO:2) includes an N-terminal

hydrophobic region that functions as a signal peptide, an extracellular domain, and a C-terminal hydrophobic region.

Computer analysis predicts that the signal peptide is cleaved after amino acid 69 of Figure 1 (SEQ ID NO:2). Cleavage of the signal peptide thus would yield a mature protein comprising amino acids 70 through 299 of Figure 1 (SEQ ID NO:2). The next most likely computer-predicted signal peptidase cleavage sites (in descending order) occur after amino acids 63 or 65 of Figure 1 (SEQ ID NO:2).

The coding region of the DNA sequence shown in Figure 1 (SEQ ID NO:1) begins with an initiation codon (ATG). A second potential initation codon is found at nucleotides 144-146 of Figure 1 (SEQ ID NO:1). Since the second (downstream) ATG is found within the region encoding the signal peptide, the mature form of the protein would be the same, regardless of which ATG functions as an initiation codon.

The extracellular domain, which follows the signal peptide, terminates at amino acid 278 of Figure 1 (SEQ ID NO:2). The amino acid sequence of the TRAIL-BP extracellular domain shows significant homology to the extracellular domains of members of the tumor necrosis factor receptor (TNF-R) family of receptors (reviewed in Smith et al., *Cell* 76:959-962, 1994).

The C-terminal hydrophobic domain comprises amino acids 279 through 299 of Figure 1 (SEQ ID NO:2). TRAIL-BP proteins containing this hydrophobic domain are attached to the cell surface.

The present invention encompasses TRAIL-BP in various forms, which may be naturally occurring or non-naturally occurring. Forms that are not naturally occurring may be produced through various techniques, such as procedures involving recombinant DNA technology. The forms of TRAIL-BP provided herein include, but are not limited to, fragments, derivatives, variants, and oligomers of TRAIL-BP, as discussed further below.

TRAIL-BP may be modified to create derivatives thereof by forming covalent or aggregative conjugates with other chemical moieties, such as glycosyl groups, lipids, phosphate, acetyl groups and the like. Covalent derivatives of TRAIL-BP may be prepared by linking the chemical moieties to functional groups on TRAIL-BP amino acid side chains or at the N-terminus or C-terminus of a TRAIL-BP polypeptide. Conjugates comprising diagnostic (detectable) or therapeutic agents attached to TRAIL-BP are contemplated herein, as discussed in more detail below.

Other derivatives of TRAIL-BP within the scope of this invention include covalent or aggregative conjugates of TRAIL-BP polypeptides with other proteins or polypeptides, such as by synthesis in recombinant culture as N-terminal or C-terminal fusions. Examples of fusion proteins are discussed below in connection with TRAIL-BP oligomers.

Further, TRAIL-BP-containing fusion proteins can comprise peptides added to facilitate purification and identification of TRAIL-BP. Such peptides include, for example, poly-His or the antigenic identification peptides described in U.S. Patent No. 5,011,912 and in Hopp et al., *Bio/Technology* 6:1204, 1988. One such peptide is the Flag® peptide, Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys (SEQ ID NO:3), which is highly antigenic and provides an epitope reversibly bound by a specific monoclonal antibody, enabling rapid assay and facile purification of expressed recombinant protein. A murine hybridoma designated 4E11 produces a monoclonal antibody that binds the Flag® peptide in the presence of certain divalent metal cations, as described in U.S. Patent 5,011,912, hereby incorporated by reference. The 4E11 hybridoma cell line has been deposited with the American Type Culture Collection under accession no. HB 9259. Monoclonal antibodies that bind the Flag® peptide are available from Eastman Kodak Co., Scientific Imaging Systems Division, New Haven, Connecticut.

Both cell membrane-bound and soluble (secreted) forms of TRAIL-BP are provided herein. Soluble TRAIL-BP may be identified (and distinguished from non-soluble membrane-bound counterparts) by separating intact cells expressing a TRAIL-BP polypeptide from the culture medium, e.g., by centrifugation, and assaying the medium (supernatant) for the presence of the desired protein. The presence of TRAIL-BP in the medium indicates that the protein was secreted from the cells and thus is a soluble form of the desired protein.

TRAIL-BP is believed to be anchored to the cell surface via glycosyl-phosphatidylinositol (GPI) linkage. GPI membrane anchors, including the chemical structure and processing thereof, are described in Ferguson, M. and A. Williams, *Ann. Rev. Biochem.*, 57:285, 1988. When initially expressed, certain proteins comprise a C-terminal hydrophobic domain that contains signals for GPI anchoring. A cleavage site is located upstream, often about 10-12 amino acids upstream of the N-terminus of the hydrophobic domain. Post-translational processing includes cleavage of the protein at this cleavage site. A GPI anchor attaches to the newly exposed C-terminal amino acid of the processed, mature protein.

Soluble forms of TRAIL-BP typically lack the C-terminal hydrophobic region that would cause retention of the protein on the cell surface. In one embodiment of the invention, a soluble TRAIL-BP polypeptide comprises the extracellular domain of the protein. Examples of soluble TRAIL-BP include, but are not limited to, mature soluble human TRAIL-BP comprising amino acids x to 278 of the Figure 1 (SEQ ID NO:2) sequence, wherein x represents an integer from 64 to 70, inclusive.

Soluble forms of TRAIL-BP possess certain advantages over the membrane-bound form of the protein. Purification of the protein from recombinant host cells is

facilitated, since the soluble proteins are secreted from the cells. Further, soluble proteins are generally more suitable for certain applications, e.g., for intravenous administration.

Naturally occurring variants of the TRAIL-BP protein of Figure 1 are provided herein. Such variants include, for example, proteins that result from alternate mRNA splicing events or from proteolytic cleavage of the TRAIL-BP protein. Alternate splicing of mRNA may, for example, yield a truncated but biologically active TRAIL-BP protein, such as a naturally occurring soluble form of the protein. Variations attributable to proteolysis include, for example, differences in the N- or C-termini upon expression in different types of host cells, due to proteolytic removal of one or more terminal amino acids from the TRAIL-BP protein (generally from 1-5 terminal amino acids). TRAIL-BP proteins in which differences in amino acid sequence are attributable to genetic polymorphism (allelic variation among individuals producing the protein) are also contemplated herein.

Regarding the discussion herein of various domains of TRAIL-BP protein, the skilled artisan will recognize that the above-described boundaries of such regions of the protein are approximate. To illustrate, the N-terminal residue of the C-terminal hydrophobic region (which may be predicted by using computer programs available for that purpose) may differ from that described above. Thus, soluble TRAIL-BP polypeptides in which the C-terminus of the extracellular domain differs from the residue so identified above are contemplated herein.

The skilled artisan will also recognize that the position(s) at which the signal peptide is cleaved may differ from that predicted by computer program, and may vary according to such factors as the type of host cells employed in expressing a recombinant TRAIL-BP polypeptide. A protein preparation may include a mixture of protein molecules having different N-terminal amino acids, resulting from cleavage of the signal peptide at more than one site. As discussed above, particular embodiments of mature TRAIL-BP proteins provided herein include, but are not limited to, proteins having the residue at position 64, 66, or 70 of Figure 1 (SEQ ID NO:2) as the N-terminal amino acid.

Other naturally occurring TRAIL-BP DNAs and polypeptides include those derived from non-human species. Homologs of the human TRAIL-BP of Figure 1, (SEQ ID NOS:1 and 2) from other mammalian species, are contemplated herein, for example. Probes based on the human DNA sequence of Figure 1 (SEQ ID NO:1) may be used to screen cDNA libraries derived from other mammalian species, using conventional cross-species hybridization techniques.

TRAIL-BP fragments are provided herein. Such fragments may be truncated at the N-terminus or C-terminus, or may lack internal residues, for example, when compared with a full length native protein. Certain fragments lack amino acid residues that are not essential for a desired biological activity, such as TRAIL binding.

TRAIL-BP fragments may be prepared by any of a number of conventional techniques. Desired peptide fragments may be chemically synthesized. An alternative approach involves generating TRAIL-BP fragments by enzymatic digestion, e.g., by treating the protein with an enzyme known to cleave proteins at sites defined by particular amino acid residues, or by digesting the DNA with suitable restriction enzymes and isolating the desired fragment. Yet another suitable technique involves isolating and amplifying a DNA fragment encoding a desired polypeptide fragment, by polymerase chain reaction (PCR). Oligonucleotides that define the desired termini of the DNA fragment are employed as the 5' and 3' primers in the PCR.

TRAIL-BP polypeptide fragments may be employed as immunogens, in generating antibodies. Certain embodiments are directed to TRAIL-BP polypeptide fragments that possess a desired biological activity, e.g., the ability to bind TRAIL. Such a fragment may be a soluble TRAIL-BP polypeptide, as described above.

In particular embodiments, the TRAIL-BP fragments include cysteine-rich repeat motifs found within the extracellular domain. A number of receptors of the TNF-R family contain cysteine-rich repeat motifs in their extracellular domains (Marsters et al., J. Biol. Chem. 267:5747-5750, 1992). These repeats are believed to be important for ligand binding. To illustrate, Marsters et al., *supra*, reported that soluble TNF-R type 1 polypeptides lacking one of the repeats exhibited a ten fold reduction in binding affinity for TNFα and TNFß; deletion of the second repeat resulted in a complete loss of detectable binding of the ligands.

The human TRAIL-BP of Figure 1 (SEQ ID NO:2) contains two such cysteine rich repeats, the first including residues 108 through 149, and the second including residues 150 through 190 of Figure 1 (SEQ ID NO:2). TRAIL-BP fragments provided herein include, but are not limited to, polypeptides that are truncated at the N-terminus and/or the C-terminus, but include the cysteine residues found within the cysteine rich repeats. Examples of such TRAIL-BP fragments include, but are not limited to, polypeptides comprising amino acids y to z of Figure 1 (SEQ ID NO:2), wherein y represents an integer from 64 to 109, and z represents an integer from 189 to 299. In particular embodiments, y is 64, 66, 70, 108, or 109, and z is 189, 190, or 278. Soluble TRAIL-BP polypeptides provided herein include, but are not limited to, fragments of the extracellular domain, wherein the fragments comprise the cysteine residues in the cysteine rich repeats.

Two expressed sequence tags (ESTs) contain regions of identity with the DNA sequence of Figure 1 (SEQ ID NO:1). The computer databank record for an EST having accession no. T71406 presents a DNA sequence 352 nucleotides in length. When the EST T71406 sequence is aligned with the TRAIL-BP DNA sequence of Figure 1 (SEQ ID NO:1), regions of identity are found between nucleotides 9 and 358 of Figure 1 (SEQ

ID NO:1). Certain of the nucleotides in EST T71406 are unidentified (i.e., are designated "N" in the databank record because their identity was unknown). The EST T71406 databank sequence also includes insertions, mismatches, and a deletion, when compared to the corresponding region of the nucleotide sequence of Figure 1 (SEQ ID NO:1).

A DNA sequence 398 nucleotides in length is presented in a computer databank for an EST having accession no. AA150849. When the EST AA150849 sequence is aligned with the TRAIL-BP DNA sequence of Figure 1 (SEQ ID NO:1), regions of identity are found between nucleotides 10 and 409 of Figure 1 (SEQ ID NO:1). However, the EST AA150849 sequence contains deletions and mismatches when compared to the corresponding nucleotide sequence of Figure 1(SEQ ID NO:1).

The EST T71406 sequence is not identical to the overlapping region of EST AA150849. Alignment of the databank sequences of these two ESTs reveals insertions and mismatches. No reading frame is identified in the databank file for either of the two ESTs. However, even if the DNA sequences set forth in the computer databank file were translated in accordance with the reading frame elucidated herein, neither EST T71406 nor AA150849 would encode a TRAIL-BP that is expected to bind TRAIL. The translates lack most of the conserved cysteine residues discussed above.

TRAIL-BP DNA sequences may vary from the native sequences disclosed herein. Due to the known degeneracy of the genetic code, wherein more than one codon can encode the same amino acid, a DNA sequence can vary from that shown in Figure 1 (SEQ ID NO:1) and still encode a TRAIL-BP protein having the amino acid sequence of Figure 1 (SEQ ID NO:2). Such variant DNA sequences may result from silent mutations (e.g., occurring during PCR amplification), or may be the product of deliberate mutagenesis of a native sequence. Thus, among the DNA sequences provided herein are native TRAIL-BP sequences (e.g., cDNA comprising the nucleotide sequence presented in Figure 1 (SEQ ID NO:1) and DNA that is degenerate as a result of the genetic code to a native TRAIL-BP DNA sequence.

Among the TRAIL-BP polypeptides provided herein are variants of native TRAIL-BP polypeptides that retain a biological activity of a native TRAIL-BP. Such variants include polypeptides that are substantially homologous to native TRAIL-BP, but which have an amino acid sequence different from that of a native TRAIL-BP because of one or more deletions, insertions or substitutions. Particular embodiments include, but are not limited to, TRAIL-BP polypeptides that comprise from one to ten deletions, insertions or substitutions of amino acid residues, when compared to a native TRAIL-BP sequence. The TRAIL-BP-encoding DNAs of the present invention include variants that differ from a native TRAIL-BP DNA sequence because of one or more deletions, insertions or substitutions, but that encode a biologically active TRAIL-BP polypeptide. One biological activity of TRAIL-BP is the ability to bind TRAIL.

Nucleic acid molecules capable of hybridizing to the DNA of Figure 1 (SEQ ID NO:1) under moderately stringent or highly stringent conditions, and which encode a biologically active TRAIL-BP, are provided herein. Such hybridizing nucleic acids include, but are not limited to, variant DNA sequences and DNA derived from non-human species, e.g., non-human mammals.

Moderately stringent conditions include conditions described in, for example, Sambrook et al, *Molecular Cloning: A Laboratory Manual*, 2nd ed., Vol. 1, pp 1.101-104, Cold Spring Harbor Laboratory Press, 1989. Conditions of moderate stringency, as defined by Sambrook et al., include use of a prewashing solution of 5X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0) and hybridization conditions of about 55_C, 5 X SSC, overnight. Highly stringent conditions include higher temperatures of hybridization and washing. One embodiment of the invention is directed to DNA sequences that will hybridize to the DNA of Figure 1 (SEQ ID NO:1) under highly stringent conditions, wherein said conditions include hybridization at 68°C followed by washing in 0.1X SSC/0.1% SDS at 63-68°C.

Certain DNAs and polypeptides provided herein comprise nucleotide or amino acid sequences, respectively, that are at least 80% identical to a native TRAIL-BP sequence. Also contemplated are embodiments in which a TRAIL-BP DNA or polypeptide comprises a sequence that is at least 90% identical, at least 95% identical, or at least 98% identical to a native TRAIL-BP sequence. The percent identity may be determined, for example, by comparing sequence information using the GAP computer program, version 6.0 described by Devereux et al. (*Nucl. Acids Res.* 12:387, 1984) and available from the University of Wisconsin Genetics Computer Group (UWGCG). The preferred default parameters for the GAP program include: (1) a unary comparison matrix (containing a value of 1 for identities and 0 for non-identities) for nucleotides, and the weighted comparison matrix of Gribskov and Burgess, *Nucl. Acids Res. 14*:6745, 1986, as described by Schwartz and Dayhoff, eds., *Atlas of Protein Sequence and Structure*, National Biomedical Research Foundation, pp. 353-358, 1979; (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps. For fragments, the percent identity is calculated by comparing the sequence of the fragment with the corresponding portion of a native TRAIL-BP.

In particular embodiments of the invention, a variant TRAIL-BP polypeptide differs in amino acid sequence from a native TRAIL-BP, but is substantially equivalent to a native TRAIL-BP in a biological activity. One example is a variant TRAIL-BP that binds TRAIL with essentially the same binding affinity as does a native TRAIL-BP. Binding affinity can be measured by conventional procedures, e.g., as described in U.S. Patent no. 5,512,457.

Variant amino acid sequences may comprise conservative substitution(s), meaning that one or more amino acid residues of a native TRAIL-BP is replaced by a different residue, but that the conservatively substituted TRAIL-BP polypeptide retains a desired biological activity of the native protein (e.g., the ability to bind TRAIL). A given amino acid may be replaced by a residue having similar physiochemical characteristics. Examples of conservative substitutions include substitution of one aliphatic residue for another, such as Ile, Val, Leu, or Ala for one another, or substitutions of one polar residue for another, such as between Lys and Arg; Glu and Asp; or Gln and Asn. Other conservative substitutions, e.g., involving substitutions of entire regions having similar hydrophobicity characteristics, are well known.

In further examples of variants, sequences are altered so that cysteine residues that are not essential for biological activity are deleted or replaced with other amino acids. Such deletion or substitution of Cys residues may reduce formation of incorrect intramolecular disulfide bridges during renaturation of the expressed protein. In one embodiment, Cys residues within the above-described cysteine rich domains remain unaltered in the TRAIL-BP variants.

Other variants are prepared by modification of adjacent dibasic amino acid residues, to enhance expression in yeast systems in which KEX2 protease activity is present. EP 212,914 discloses the use of site-specific mutagenesis to inactivate KEX2 protease processing sites in a protein. KEX2 protease processing sites are inactivated by deleting, adding or substituting residues to alter Arg-Arg, Arg-Lys, and Lys-Arg pairs to eliminate the occurrence of these adjacent basic residues. The human TRAIL-BP of Figure 1 (SEQ ID NO:2) contains one such adjacent basic residue pair, at amino acids 166-167. Lys-Lys pairings are considerably less susceptible to KEX2 cleavage, and conversion of Arg-Lys or Lys-Arg to Lys-Lys represents a conservative and preferred approach to inactivating KEX2 sites.

In still other variants, N-glycosylation sites in a native TRAIL-BP are inactivated. N-glycosylation sites can be modified to preclude glycosylation, allowing expression of a more homogeneous, reduced carbohydrate analog in mammalian and yeast expression systems. N-glycosylation sites in eukaryotic polypeptides are characterized by an amino acid triplet Asn-X-Y, wherein X is any amino acid except Pro and Y is Ser or Thr. The mature form of the human TRAIL-BP protein of Figure 1 (SEQ ID NO:2) comprises five such triplets, at amino acids 117-119, 180-182, 196-198, 209-211, and 224-226 of Figure 1(SEQ ID NO:2). Appropriate substitutions, additions or deletions to the nucleotide sequence encoding these triplets will result in prevention of attachment of carbohydrate residues to the Asn side chain. Alteration of a single nucleotide, chosen so that Asn is replaced by a different amino acid, for example, is sufficient to inactivate an N-glycosylation site. Known procedures for inactivating N-glycosylation sites in proteins

include those described in U.S. Patent 5,071,972 and EP 276,846, hereby incorporated by reference.

The calculated molecular weight for a mature protein containing residues 70 to 299 of Figure 1 (SEQ ID NO:2) is about 24.3 kilodaltons. The skilled artisan will recognize that the molecular weight of particular preparations of TRAIL-BP protein may differ, according to such factors as the degree of glycosylation. The glycosylation pattern of a particular preparation of TRAIL-BP may vary according to the type of cells in which the protein is expressed, for example, and a given preparation may include multiple differentially glycosylated species of the protein. TRAIL-BP polypeptides with or without associated native-pattern glycosylation are provided herein. Expression of TRAIL-BP polypeptides in bacterial expression systems, such as *E. coli*, provides non-glycosylated molecules. Further, N-glycosylation sites in the native protein may be inactivated, as discussed above.

TRAIL-BP polypeptides, including variants and fragments thereof, can be tested for biological activity in any suitable assay. The ability of a TRAIL-BP polypeptide to bind TRAIL can be confirmed in conventional binding assays, examples of which are described below.

Expression Systems

The present invention also provides recombinant cloning and expression vectors containing TRAIL-BP DNA, as well as host cells containing the recombinant vectors. Expression vectors comprising TRAIL-BP DNA may be used to prepare TRAIL-BP polypeptides encoded by the DNA. A method for producing TRAIL-BP polypeptides comprises culturing host cells containing a recombinant expression vector encoding TRAIL-BP, under conditions that allow expression of TRAIL-BP, then recovering the expressed TRAIL-BP polypeptides from the culture. The skilled artisan will recognize that the procedure for purifying the expressed TRAIL-BP will vary according to such factors as the type of host cells employed, and whether the TRAIL-BP is cell membrane-bound or a soluble form that is secreted from the host cell.

Any suitable expression system may be employed. The vectors include a DNA encoding a TRAIL-BP polypeptide, operably linked to suitable transcriptional or translational regulatory nucleotide sequences, such as those derived from a mammalian, microbial, viral, or insect gene. Examples of regulatory sequences include transcriptional promoters, operators, or enhancers, an mRNA ribosomal binding site, and appropriate sequences which control transcription and translation initiation and termination. Nucleotide sequences are operably linked when the regulatory sequence functionally

relates to the TRAIL-BP DNA sequence. Thus, a promoter nucleotide sequence is operably linked to an TRAIL-BP DNA sequence if the promoter nucleotide sequence controls the transcription of the TRAIL-BP DNA sequence. An origin of replication that confers the ability to replicate in the desired host cells, and a selection gene by which transformants are identified, are generally incorporated into the expression vector.

In addition, a sequence encoding an appropriate signal peptide (native or heterologous) can be incorporated into expression vectors. A DNA sequence for a signal peptide (secretory leader) may be fused in frame to the TRAIL-BP sequence so that the TRAIL-BP is initially translated as a fusion protein comprising the signal peptide. A signal peptide that is functional in the intended host cells promotes extracellular secretion of the TRAIL-BP polypeptide. The signal peptide is cleaved from the TRAIL-BP polypeptide upon secretion of TRAIL-BP from the cell.

Suitable host cells for expression of TRAIL-BP polypeptides include prokaryotes, yeast or higher eukaryotic cells. Mammalian or insect cells are generally preferred for use as host cells. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described, for example, in Pouwels et al. *Cloning Vectors: A Laboratory Manual,* Elsevier, New York, (1985). Cell-free translation systems could also be employed to produce TRAIL-BP polypeptides using RNAs derived from DNA constructs disclosed herein.

Prokaryotes include gram negative or gram positive organisms, for example, *E. coli* or *Bacilli.* Suitable prokaryotic host cells for transformation include, for example, *E. coli, Bacillus subtilis, Salmonella typhimurium,* and various other species within the genera *Pseudomonas, Streptomyces,* and *Staphylococcus.* In a prokaryotic host cell, such as *E. coli,* a TRAIL-BP polypeptide may include an N-terminal methionine residue to facilitate expression of the recombinant polypeptide in the prokaryotic host cell. The N-terminal Met may be cleaved from the expressed recombinant TRAIL-BP polypeptide.

Expression vectors for use in prokaryotic host cells generally comprise one or more phenotypic selectable marker genes. A phenotypic selectable marker gene is, for example, a gene encoding a protein that confers antibiotic resistance or that supplies an autotrophic requirement. Examples of useful expression vectors for prokaryotic host cells include those derived from commercially available plasmids such as the cloning vector pBR322 (ATCC 37017). pBR322 contains genes for ampicillin and tetracycline resistance and thus provides simple means for identifying transformed cells. An appropriate promoter and a TRAIL-BP DNA sequence are inserted into the pBR322

vector. Other commercially available vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and pGEM1 (Promega Biotec, Madison, WI, USA).

Promoter sequences commonly used for recombinant prokaryotic host cell expression vectors include β-lactamase (penicillinase), lactose promoter system (Chang et al., *Nature 275*:615, 1978; and Goeddel et al., *Nature 281*:544, 1979), tryptophan (trp) promoter system (Goeddel et al., *Nucl. Acids Res. 8*:4057, 1980; and EP-A-36776) and tac promoter (Maniatis, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, p. 412, 1982). A particularly useful prokaryotic host cell expression system employs a phage λ P_L promoter and a cI857ts thermolabile repressor sequence. Plasmid vectors available from the American Type Culture Collection which incorporate derivatives of the λ P_L promoter include plasmid pHUB2 (resident in *E. coli* strain JMB9, ATCC 37092) and pPLc28 (resident in *E. coli* RR1, ATCC 53082).

TRAIL-BP alternatively may be expressed in yeast host cells, preferably from the *Saccharomyces* genus (e.g., *S. cerevisiae*). Other genera of yeast, such as *Pichia* or *Kluyveromyces*, may also be employed. Yeast vectors will often contain an origin of replication sequence from a 2μ yeast plasmid, an autonomously replicating sequence (ARS), a promoter region, sequences for polyadenylation, sequences for transcription termination, and a selectable marker gene. Suitable promoter sequences for yeast vectors include, among others, promoters for metallothionein, 3-phosphoglycerate kinase (Hitzeman et al., *J. Biol. Chem. 255*:2073, 1980) or other glycolytic enzymes (Hess et al., *J. Adv. Enzyme Reg. 7*:149, 1968; and Holland et al., *Biochem. 17*:4900, 1978), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phospho-glucose isomerase, and glucokinase. Other suitable vectors and promoters for use in yeast expression are further described in Hitzeman, EPA-73,657. Another alternative is the glucose-repressible ADH2 promoter described by Russell et al. (*J. Biol. Chem. 258*:2674, 1982) and Beier et al. (*Nature 300*:724, 1982). Shuttle vectors replicable in both yeast and *E. coli* may be constructed by inserting DNA sequences from pBR322 for selection and replication in *E. coli* (Amp^r gene and origin of replication) into the above-described yeast vectors.

The yeast α-factor leader sequence may be employed to direct secretion of the TRAIL polypeptide. The α-factor leader sequence is often inserted between the promoter

sequence and the structural gene sequence. *See*, e.g., Kurjan et al., *Cell 30*:933, 1982 and Bitter et al., *Proc. Natl. Acad. Sci. USA 81*:5330, 1984. Other leader sequences suitable for facilitating secretion of recombinant polypeptides from yeast hosts are known to those of skill in the art. A leader sequence may be modified near its 3' end to contain one or more restriction sites. This will facilitate fusion of the leader sequence to the structural gene.

Yeast transformation protocols are known to those of skill in the art. One such protocol is described by Hinnen et al., *Proc. Natl. Acad. Sci. USA 75*:1929, 1978. The Hinnen et al. protocol selects for Trp^+ transformants in a selective medium, wherein the selective medium consists of 0.67% yeast nitrogen base, 0.5% casamino acids, 2% glucose, 10 μg/ml adenine and 20 μg/ml uracil.

Yeast host cells transformed by vectors containing an ADH2 promoter sequence may be grown for inducing expression in a "rich" medium. An example of a rich medium is one consisting of 1% yeast extract, 2% peptone, and 1% glucose supplemented with 80 μg/ml adenine and 80 μg/ml uracil. Derepression of the ADH2 promoter occurs when glucose is exhausted from the medium.

Mammalian or insect host cell culture systems also may be employed to express recombinant TRAIL-BP polypeptides. Baculovirus systems for production of heterologous proteins in insect cells are reviewed by Luckow and Summers, *Bio/Technology* 6:47 (1988). Established cell lines of mammalian origin also may be employed. Examples of suitable mammalian host cell lines include the COS-7 line of monkey kidney cells (ATCC CRL 1651) (Gluzman et al., *Cell 23*:175, 1981), L cells, C127 cells, 3T3 cells (ATCC CCL 163), Chinese hamster ovary (CHO) cells, HeLa cells, BHK (ATCC CRL 10) cells, and the CV1/EBNA cell line (ATCC CRL 10478) that was derived from the African green monkey kidney cell line CV1 (ATCC CCL 70) as described by McMahan et al. (*EMBO J.* 10: 2821, 1991).

Transcriptional and translational control sequences for mammalian host cell expression vectors may be excised from viral genomes. Commonly used promoter sequences and enhancer sequences are derived from Polyoma virus, Adenovirus 2, Simian Virus 40 (SV40), and human cytomegalovirus. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early and late promoter, enhancer, splice, and polyadenylation sites may be used to provide other genetic elements for expression of a structural gene sequence in a mammalian host cell. Viral early and late promoters are

particularly useful because both are easily obtained from a viral genome as a fragment which may also contain a viral origin of replication (Fiers et al., *Nature 273*:113, 1978). Smaller or larger SV40 fragments may also be used, provided the approximately 250 bp sequence extending from the *Hind* III site toward the *Bgl* I site located in the SV40 viral origin of replication site is included.

Expression vectors for use in mammalian host cells can be constructed as disclosed by Okayama and Berg (*Mol. Cell. Biol. 3*:280, 1983), for example. A useful system for stable high level expression of mammalian cDNAs in C127 murine mammary epithelial cells can be constructed substantially as described by Cosman et al. (*Mol. Immunol.* 23:935, 1986). A high expression vector, PMLSV N1/N4, described by Cosman et al., *Nature 312*:768, 1984 has been deposited as ATCC 39890. Additional mammalian expression vectors are described in EP-A-0367566, and in WO 91/18982. As one alternative, the vector may be derived from a retrovirus.

Regarding signal peptides that may be employed in producing TRAIL-BP, the native signal peptide of TRAIL-BP may be replaced by a heterologous signal peptide or leader sequence, if desired. The choice of signal peptide or leader may depend on factors such as the type of host cells in which the recombinant TRAIL-BP is to be produced. To illustrate, examples of heterologous signal peptides that are functional in mammalian host cells include the signal sequence for interleukin-7 (IL-7) described in United States Patent 4,965,195, the signal sequence for interleukin-2 receptor described in Cosman et al., *Nature* 312:768 (1984); the interleukin-4 receptor signal peptide described in EP 367,566; the type I interleukin-1 receptor signal peptide described in U.S. Patent 4,968,607; and the type II interleukin-1 receptor signal peptide described in EP 460,846.

Purified Protein

TRAIL-BP polypeptides of the present invention may be produced by recombinant expression systems as described above, or purified from naturally occurring cells. TRAIL-BP may be purified by any of a number of suitable methods, which may employ conventional protein purification techniques. As is known to the skilled artisan, procedures for purifying a given protein are chosen according to such factors as the types of contaminants that are to be removed, which may vary according to the particular cells in which the TRAIL-BP is expressed. For recombinant proteins, other considerations include the particular expression systems employed and whether or not the desired protein is secreted into the culture medium.

In one method, cells expressing the protein are disrupted by any of the numerous known techniques, including freeze-thaw cycling, sonication, mechanical disruption, or by use of cell lysing agents. Alternatively, a soluble TRAIL-BP may be expressed and secreted from the cell. The subsequent purification process may include affinity chromatography, e.g., employing a chromatography matrix containing TRAIL. The chromatography matrix may instead comprise an antibody that binds TRAIL-BP. The TRAIL-BP polypeptides can be recovered from an affinity chromatography column using conventional techniques (e.g., elution in a high salt buffer), then dialyzed into a lower salt buffer for use.

In one approach, when an expression system that secretes the recombinant protein is employed, the culture medium first may be concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate can be applied to a purification matrix such as a gel filtration matrix. Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other support materials commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. Sulfopropyl groups are preferred. In addition, one or more reversed-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, (e.g., silica gel having pendant methyl or other aliphatic groups) can be employed. Some or all of the foregoing purification steps, in various combinations, may be employed.

Recombinant protein produced in bacterial culture can be isolated by initial disruption of the host cells, centrifugation, extraction from cell pellets if an insoluble polypeptide, or from the supernatant fluid if a soluble polypeptide, followed by one or more concentration, salting-out, ion exchange, affinity purification or size exclusion chromatography steps. Finally, RP-HPLC can be employed for final purification steps. Microbial cells can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

In yeast host cells, TRAIL-BP is preferably expressed as a secreted polypeptide, to simplify purification. Recombinant polypeptides secreted from a yeast host cell fermentation can be purified by methods analogous to those disclosed by Urdal et al. (*J. Chromatog.* 296:171, 1984). Urdal et al. describe two sequential, reversed-phase HPLC steps for purification of recombinant human IL-2 on a preparative HPLC column.

The desired degree of purity depends on the intended use of the protein. A relatively high degree of purity is desired when the protein is to be administered *in vivo*, for example. Advantageously, TRAIL-BP polypeptides are purified such that no protein

bands corresponding to other (non-TRAIL-BP) proteins are detectable upon analysis by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). One skilled in the pertinent field will recognize that multiple bands corresponding to TRAIL-BP protein may be visualized by SDS-PAGE, due to differential glycosylation, differential post-translational processing, and the like. TRAIL-BP most preferably is purified to substantial homogeneity, as indicated by a single protein band upon analysis by SDS-PAGE. The protein band may be visualized by silver staining, Coomassie blue staining, or (if the protein is radiolabeled) by autoradiography.

Oligomeric Forms of TRAIL-BP

Encompassed by the present invention are oligomers that contain TRAIL-BP polypeptides. TRAIL-BP oligomers may be in the form of covalently-linked or non-covalently-linked dimers, trimers, or higher oligomers.

One embodiment of the invention is directed to oligomers comprising multiple TRAIL-BP polypeptides joined *via* covalent or non-covalent interactions between peptide moieties fused to the TRAIL-BP polypeptides. Such peptides may be peptide linkers (spacers), or peptides that have the property of promoting oligomerization. Leucine zippers and certain polypeptides derived from antibodies are among the peptides that can promote oligomerization of TRAIL-BP polypeptides attached thereto, as described in more detail below.

In particular embodiments, the oligomers comprise from two to four TRAIL-BP polypeptides. The TRAIL-BP moieties of the oligomer may be soluble polypeptides, as described above.

As one alternative, a TRAIL-BP oligomer is prepared using polypeptides derived from immunoglobulins. Preparation of fusion proteins comprising certain heterologous polypeptides fused to various portions of antibody-derived polypeptides (including the Fc domain) has been described, e.g., by Ashkenazi et al. (*PNAS USA* 88:10535, 1991); Byrn et al. (*Nature* 344:677, 1990); and Hollenbaugh and Aruffo ("Construction of Immunoglobulin Fusion Proteins", in *Current Protocols in Immunology*, Suppl. 4, pages 10.19.1 - 10.19.11, 1992).

One embodiment of the present invention is directed to a TRAIL-BP dimer comprising two fusion proteins created by fusing TRAIL-BP to the Fc region of an antibody. The TRAIL-BP moiety preferably is a soluble polypeptide. A gene fusion encoding the TRAIL-BP/Fc fusion protein is inserted into an appropriate expression vector. TRAIL-BP/Fc fusion proteins are expressed in host cells transformed with the recombinant expression vector, and allowed to assemble much like antibody molecules, whereupon interchain disulfide bonds form between the Fc moieties to yield divalent TRAIL-BP.

Provided herein are fusion proteins comprising a TRAIL-BP polypeptide fused to an Fc polypeptide derived from an antibody. DNA encoding such fusion proteins, as well as dimers containing two fusion proteins joined *via* disulfide bonds between the Fc moieties thereof, are also provided. The term "Fc polypeptide" as used herein includes native and mutein forms of polypeptides derived from the Fc region of an antibody. Truncated forms of such polypeptides containing the hinge region that promotes dimerization are also included.

One suitable Fc polypeptide, described in PCT application WO 93/10151 (hereby incorporated by reference), is a single chain polypeptide extending from the N-terminal hinge region to the native C-terminus of the Fc region of a human IgG1 antibody. Another useful Fc polypeptide is the Fc mutein described in U.S. Patent 5,457,035 and in Baum et al., (*EMBO J.* 13:3992-4001, 1994). The amino acid sequence of this mutein is identical to that of the native Fc sequence presented in WO 93/10151, except that amino acid 19 has been changed from Leu to Ala, amino acid 20 has been changed from Leu to Glu, and amino acid 22 has been changed from Gly to Ala. The mutein exhibits reduced affinity for Fc receptors.

In other embodiments, TRAIL-BP is substituted for the variable portion of an antibody heavy or light chain. If fusion proteins are made with both heavy and light chains of an antibody, it is possible to form a TRAIL-BP oligomer with as many as four TRAIL-BP extracellular regions.

Alternatively, the oligomer is a fusion protein comprising multiple TRAIL-BP polypeptides, with or without peptide linkers (spacer peptides). Among the suitable peptide linkers are those described in U.S. Patents 4,751,180 and 4,935,233, which are hereby incorporated by reference. A DNA sequence encoding a desired peptide linker may be inserted between, and in the same reading frame as, the DNA sequences encoding TRAIL-BP, using any suitable conventional technique. In one approach, a chemically synthesized oligonucleotide encoding the linker is ligated between sequences encoding TRAIL-BP. In particular embodiments, a fusion protein comprises from two to four soluble TRAIL-BP polypeptides, separated by peptide linkers.

Another method for preparing oligomeric TRAIL-BP involves use of a leucine zipper. Leucine zipper domains are peptides that promote oligomerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., *Science* 240:1759, 1988), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble oligomeric proteins are described in PCT application WO 94/10308 (hereby incorporated by reference), and the leucine zipper derived from lung surfactant protein D (SPD) described in Hoppe et al. (*FEBS Letters*

344:191, 1994), hereby incorporated by reference. The use of a modified leucine zipper that allows for stable trimerization of a heterologous protein fused thereto is described in Fanslow et al. (*Semin. Immunol.* 6:267-278, 1994). Recombinant fusion proteins comprising a soluble TRAIL-BP polypeptide fused to a leucine zipper peptide are expressed in suitable host cells, and the soluble oligomeric TRAIL-BP that forms is recovered from the culture supernatant.

Oligomeric TRAIL-BP has the property of bivalent, trivalent, etc. binding sites for TRAIL. The above-described fusion proteins comprising Fc moieties (and oligomers formed therefrom) offer the advantage of facile purification by affinity chromatography over Protein A or Protein G columns. DNA sequences encoding oligomeric TRAIL-BP, or encoding fusion proteins useful in preparing TRAIL-BP oligomers, are provided herein.

Assays

TRAIL-BP proteins (including fragments, variants, oligomers, and other forms of TRAIL-BP) may be tested for the ability to bind TRAIL in any suitable assay, such as a conventional binding assay. To illustrate, a soluble TRAIL-BP may be labeled with a detectable reagent (e.g., a radionuclide, chromophore, enzyme that catalyzes a colorimetric or fluorometric reaction, and the like). The labeled TRAIL-BP is contacted with cells expressing TRAIL. The cells then are washed to remove unbound labeled TRAIL-BP, and the presence of cell-bound label is determined by a suitable technique, chosen according to the nature of the label.

One example of a binding assay procedure is as follows. A recombinant expression vector containing TRAIL cDNA is constructed, e.g., as described in in PCT application WO 97/01633, hereby incorporated by reference. DNA and amino acid sequence information for human and mouse TRAIL is presented in WO 97/01633. TRAIL comprises an N-terminal cytoplasmic domain, a transmembrane region, and a C-terminal extracellular domain. CV1-EBNA-1 cells in 10 cm^2 dishes are transfected with the recombinant expression vector. CV-1/EBNA-1 cells (ATCC CRL 10478) constitutively express EBV nuclear antigen-1 driven from the CMV immediate-early enhancer/promoter. CV1-EBNA-1 was derived from the African Green Monkey kidney cell line CV-1 (ATCC CCL 70), as described by McMahan et al. (*EMBO J.* 10:2821, 1991).

The transfected cells are cultured for 24 hours, and the cells in each dish then are split into a 24-well plate. After culturing an additional 48 hours, the transfected cells (about 4 x 10^4 cells/well) are washed with BM-NFDM, which is binding medium (RPMI 1640 containing 25 mg/ml bovine serum albumin, 2 mg/ml sodium azide, 20 mM Hepes pH 7.2) to which 50 mg/ml nonfat dry milk has been added. The cells then are incubated

for 1 hour at 37°C with various concentrations of a soluble TRAIL-BP/Fc fusion protein. Cells then are washed and incubated with a constant saturating concentration of a ^{125}I-mouse anti-human IgG in binding medium, with gentle agitation for 1 hour at 37°C. After extensive washing, cells are released *via* trypsinization.

The mouse anti-human IgG employed above is directed against the Fc region of human IgG and can be obtained from Jackson Immunoresearch Laboratories, Inc., West Grove, PA. The antibody is radioiodinated using the standard chloramine-T method. The antibody will bind to the Fc portion of any TRAIL-BP/Fc protein that has bound to the cells. In all assays, non-specific binding of ^{125}I-antibody is assayed in the absence of TRAIL-BP/Fc, as well as in the presence of TRAIL-BP/Fc and a 200-fold molar excess of unlabeled mouse anti-human IgG antibody.

Cell-bound ^{125}I-antibody is quantified on a Packard Autogamma counter. Affinity calculations (Scatchard, *Ann. N.Y. Acad. Sci.* 51:660, 1949) are generated on RS/1 (BBN Software, Boston, MA) run on a Microvax computer.

Another type of suitable binding assay is a competitive binding assay. To illustrate, biological activity of a TRAIL-BP variant may be determined by assaying for the variant's ability to compete with a native TRAIL-BP for binding to TRAIL.

Competitive binding assays can be performed by conventional methodology. Reagents that may be employed in competitive binding assays include radiolabeled TRAIL-BP and intact cells expressing TRAIL (endogenous or recombinant) on the cell surface. For example, a radiolabeled soluble TRAIL-BP fragment can be used to compete with a soluble TRAIL-BP variant for binding to cell surface TRAIL. Instead of intact cells, one could substitute a soluble TRAIL/Fc fusion protein bound to a solid phase through the interaction of Protein A or Protein G (on the solid phase) with the Fc moiety. Chromatography columns that contain Protein A and Protein G include those available from Pharmacia Biotech, Inc., Piscataway, NJ. In one alternative, LZ-TRAIL (a fusion protein comprising a leucine zipper peptide fused to a soluble TRAIL polypeptide; see example 1) is employed instead of TRAIL/Fc. The LZ-TRAIL may be attached to a Protein A or Protein G column via a monoclonal antibody specific for the leucine zipper peptide.

Another type of competitive binding assay utilizes radiolabeled soluble TRAIL, such as a soluble TRAIL/Fc fusion protein, and intact cells expressing TRAIL-BP. A soluble leucine zipper/TRAIL fusion protein may be employed in place of a TRAIL/Fc fusion protein. Qualitative results can be obtained by competitive autoradiographic plate binding assays, while Scatchard plots (Scatchard, *Ann. N.Y. Acad. Sci.* 51:660, 1949) may be utilized to generate quantitative results.

Another type of assay for biological activity involves testing a TRAIL-BP polypeptide (preferably a soluble TRAIL-BP) for the ability to block TRAIL-mediated apoptosis of target cells, such as the human leukemic T-cell line known as Jurkat cells, for example. TRAIL-mediated apoptosis of the cell line designated Jurkat clone E6-1 (ATCC TIB 152) is demonstrated in assay procedures described in PCT application WO 97/01633, hereby incorporated by reference.

Uses of TRAIL-BP

Uses of TRAIL-BP include, but are not limited to, the following. Certain of these uses of TRAIL-BP flow from its ability to bind TRAIL.

TRAIL-BP finds use as a protein purification reagent. TRAIL-BP polypeptides may be attached to a suitable support material (generally an insoluble matrix) and used to purify TRAIL proteins by affinity chromatography. In particular embodiments, a TRAIL-BP polypeptide (in any form described herein that is capable of binding TRAIL) is attached to a solid support by conventional procedures. As one example, chromatography columns containing functional groups that will react with functional groups on amino acid side chains of proteins are available (Pharmacia Biotech, Inc., Piscataway, NJ). In an alternative, a TRAIL-BP/Fc protein is attached to Protein A- or Protein G-containing chromatography columns through interaction with the Fc moiety.

TRAIL-BP proteins also find use in measuring the biological activity of TRAIL proteins in terms of their binding affinity for TRAIL-BP. TRAIL-BP proteins thus may be employed by those conducting "quality assurance" studies, e.g., to monitor shelf life and stability of TRAIL protein under different conditions. To illustrate, TRAIL-BP may be employed in a binding affinity study to measure the biological activity of a TRAIL protein that has been stored at different temperatures, or produced in different cell types. TRAIL-BP also may be used to determine whether biological activity is retained after modification of a TRAIL protein (e.g., chemical modification, truncation, mutation, etc.). The binding affinity of the modified TRAIL protein for TRAIL-BP is compared to that of an unmodified TRAIL protein to detect any adverse impact of the modifications on biological activity of TRAIL. The biological activity of a TRAIL protein preparation thus can be ascertained before it is used in a research study, for example.

TRAIL-BP also finds use in purifying or identifying cells that express TRAIL on the cell surface. TRAIL-BP polypeptides are bound to a solid phase such as a column chromatography matrix or a similar suitable substrate. For example, magnetic microspheres can be coated with TRAIL-BP and held in an incubation vessel through a magnetic field. Suspensions of cell mixtures containing TRAIL-expressing cells are contacted with the solid phase having TRAIL-BP thereon. Cells expressing TRAIL on the cell surface bind to the fixed TRAIL-BP, and unbound cells then are washed away.

Alternatively, TRAIL-BP can be conjugated to a detectable moiety, then incubated with cells to be tested for TRAIL expression. After incubation, unbound labeled TRAIL-BP is removed and the presence or absence of the detectable moiety on the cells is determined.

In a further alternative, mixtures of cells suspected of containing TRAIL$^+$ cells are incubated with biotinylated TRAIL-BP. Incubation periods are typically at least one hour in duration to ensure sufficient binding. The resulting mixture then is passed through a column packed with avidin-coated beads, whereby the high affinity of biotin for avidin promotes binding of the desired cells to the beads. Procedures for using avidin-coated beads are known (see Berenson, et al. *J. Cell. Biochem.*, 10D:239, 1986). Washing to remove unbound material, and the release of the bound cells, are performed using conventional methods.

TRAIL-BP polypeptides also find use as carriers for delivering agents attached thereto to cells bearing TRAIL. Cells expressing TRAIL include those identified in Wiley et al. (*Immunity*, 3:673-682, 1995). TRAIL-BP proteins thus can be used to deliver diagnostic or therapeutic agents to such cells (or to other cell types found to express TRAIL on the cell surface) in *in vitro* or *in vivo* procedures.

Detectable (diagnostic) and therapeutic agents that may be attached to a TRAIL-BP polypeptide include, but are not limited to, toxins, other cytotoxic agents, drugs, radionuclides, chromophores, enzymes that catalyze a colorimetric or fluorometric reaction, and the like, with the particular agent being chosen according to the intended application. Among the toxins are ricin, abrin, diphtheria toxin, *Pseudomonas aeruginosa* exotoxin A, ribosomal inactivating proteins, mycotoxins such as trichothecenes, and derivatives and fragments (e.g., single chains) thereof. Radionuclides suitable for diagnostic use include, but are not limited to, ^{123}I, ^{131}I, ^{99m}Tc, ^{111}In, and ^{76}Br. Examples of radionuclides suitable for therapeutic use are ^{131}I, ^{211}At, ^{77}Br, ^{186}Re, ^{188}Re, ^{212}Pb, ^{212}Bi, ^{109}Pd, ^{64}Cu, and ^{67}Cu.

Such agents may be attached to the TRAIL-BP by any suitable conventional procedure. TRAIL-BP, being a protein, comprises functional groups on amino acid side chains that can be reacted with functional groups on a desired agent to form covalent bonds, for example. Alternatively, the protein or agent may be derivatized to generate or attach a desired reactive functional group. The derivatization may involve attachment of one of the bifunctional coupling reagents available for attaching various molecules to proteins (Pierce Chemical Company, Rockford, Illinois). A number of techniques for radiolabeling proteins are known. Radionuclide metals may be attached to TRAIL-BP by using a suitable bifunctional chelating agent, for example.

Conjugates comprising TRAIL-BP and a suitable diagnostic or therapeutic agent (preferably covalently linked) are thus prepared. The conjugates are administered or otherwise employed in an amount appropriate for the particular application.

TRAIL-BP DNA and polypeptides of the present invention may be used in developing treatments for any disorder mediated (directly or indirectly) by defective, or insufficient amounts of, TRAIL-BP. TRAIL-BP polypeptides may be administered to a mammal afflicted with such a disorder. Alternatively, a gene therapy approach may be taken. Disclosure herein of native TRAIL-BP nucleotide sequences permits the detection of defective TRAIL-BP genes, and the replacement thereof with normal TRAIL-BP-encoding genes. Defective genes may be detected in *in vitro* diagnostic assays, and by comparision of a native TRAIL-BP nucleotide sequence disclosed herein with that of a TRAIL-BP gene derived from a person suspected of harboring a defect in this gene.

Another use of the protein of the present invention is as a research tool for studying the biological effects that result from inhibiting TRAIL/TRAIL-BP interactions on different cell types. TRAIL-BP polypeptides also may be employed in *in vitro* assays for detecting TRAIL or TRAIL-BP or the interactions thereof.

A purified TRAIL-BP polypeptide may be used to inhibit binding of TRAIL to endogenous cell surface TRAIL receptors. Certain ligands of the TNF family (of which TRAIL is a member) have been reported to bind to more than one distinct cell surface receptor protein. TRAIL likewise may bind to multiple cell surface proteins. A receptor protein designated DR4 that reportedly binds TRAIL, but is distinct from the TRAIL-BP of the present invention, is described in Pan et al. (*Science* 276:111-113, 1997; hereby incorporated by reference). By binding TRAIL, soluble TRAIL-BP polypeptides of the present invention may be employed to inhibit the binding of TRAIL not only to cell surface TRAIL-BP, but also to TRAIL receptor proteins that are distinct from TRAIL-BP.

TRAIL-BP may be used to inhibit a biological activity of TRAIL, in *in vitro* or *in vivo* procedures. By inhibiting binding of TRAIL to cell surface receptors, TRAIL-BP also inhibits biological effects that result from the binding of TRAIL to endogenous receptors. Various forms of TRAIL-BP may be employed, including, for example, the above-described TRAIL-BP fragments, oligomers, derivatives, and variants that are capable of binding TRAIL. In a preferred embodiment, a soluble TRAIL-BP is employed to inhibit a biological activity of TRAIL, e.g., to inhibit TRAIL-mediated apoptosis of cells susceptible to such apoptosis.

TRAIL-BP may be administered to a mammal to treat a TRAIL-mediated disorder. Such TRAIL-mediated disorders include conditions caused (directly or indirectly) or exacerbated by TRAIL.

TRAIL-BP may be useful for treating thrombotic microangiopathies. One such disorder is thrombotic thrombocytopenic purpura (TTP) (Kwaan, H.C., *Semin. Hematol.*,

24:71, 1987; Thompson et al., *Blood*, 80:1890, 1992). Increasing TTP-associated mortality rates have been reported by the U.S. Centers for Disease Control (Torok et al., *Am. J. Hematol.* 50:84, 1995).

Plasma from patients afflicted with TTP (including HIV^+ and HIV^- patients) induces apoptosis of human endothelial cells of dermal microvascular origin, but not large vessel origin (Laurence et al., *Blood*, 87:3245, April 15, 1996). Plasma of TTP patients thus is thought to contain one or more factors that directly or indirectly induce apoptosis. As described in PCT application WO 97/01633 (hereby incorporated by reference), TRAIL is present in the serum of TTP patients, and may play a role in inducing apoptosis of microvascular endothelial cells.

Another thrombotic microangiopathy is hemolytic-uremic syndrome (HUS) (Moake, J.L., *Lancet*, 343:393, 1994; Melnyk et al., (*Arch. Intern. Med.*, 155:2077, 1995; Thompson et al., *supra*). One embodiment of the invention is directed to use of TRAIL-BP to treat the condition that is often referred to as "adult HUS" (even though it can strike children as well). A disorder known as childhood/diarrhea-associated HUS differs in etiology from adult HUS.

Other conditions characterized by clotting of small blood vessels may be treated using TRAIL-BP. Such conditions include but are not limited to the following. Cardiac problems seen in about 5-10% of pediatric AIDS patients are believed to involve clotting of small blood vessels. Breakdown of the microvasculature in the heart has been reported in multiple sclerosis patients. As a further example, treatment of systemic lupus erythematosus (SLE) is contemplated.

In one embodiment, a patient's blood or plasma is contacted with TRAIL-BP *ex vivo*. The TRAIL-BP may be bound to a suitable chromatography matrix by conventional procedures. The patient's blood or plasma flows through a chromatography column containing TRAIL-BP bound to the matrix, before being returned to the patient. The immobilized TRAIL-BP binds TRAIL, thus removing TRAIL protein from the patient's blood.

Alternatively, TRAIL-BP may be administered *in vivo* to a patient afflicted with a thrombotic microangiopathy. In one embodiment, a soluble form of TRAIL-BP is administered to the patient.

The present invention thus provides a method for treating a thrombotic microangiopathy, involving use of an effective amount of TRAIL-BP. A TRAIL-BP polypeptide may be employed in *in vivo* or *ex vivo* procedures, to inhibit TRAIL-mediated damage to (e.g., apoptosis of) microvascular endothelial cells.

TRAIL-BP may be employed in conjunction with other agents useful in treating a particular disorder. In an *in vitro* study reported by Laurence et al. (*Blood* 87:3245, 1996), some reduction of TTP plasma-mediated apoptosis of microvascular endothelial

cells was achieved by using an anti-Fas blocking antibody, aurintricarboxylic acid, or normal plasma depleted of cryoprecipitate.

Thus, a patient may be treated with an agent that inhibits Fas-ligand-mediated apoptosis of endothelial cells, in combination with an agent that inhibits TRAIL-mediated apoptosis of endothelial cells. In one embodiment, TRAIL-BP and an anti-FAS blocking antibody are both administered to a patient afflicted with a disorder characterized by thrombotic microangiopathy, such as TTP or HUS. Examples of blocking monoclonal antibodies directed against Fas antigen (CD95) are described in PCT application publication number WO 95/10540, hereby incorporated by reference.

Another embodiment of the present invention is directed to the use of TRAIL-BP to reduce TRAIL-mediated death of T cells in HIV-infected patients. The role of T cell apoptosis in the development of AIDS has been the subject of a number of studies (see, for example, Meyaard et al., *Science* 257:217-219, 1992; Groux et al., *J Exp. Med.*, 175:331, 1992; and Oyaizu et al., in *Cell Activation and Apoptosis in HIV Infection*, Andrieu and Lu, Eds., Plenum Press, New York, 1995, pp. 101-114). Certain investigators have studied the role of Fas-mediated apoptosis; the involvement of interleukin-1ß-converting enzyme (ICE) also has been explored (Estaquier et al., *Blood* 87:4959-4966, 1996; Mitra et al., *Immunology* 87:581-585, 1996; Katsikis et al., *J. Exp. Med.* 181:2029-2036, 1995). It is possible that T cell apoptosis occurs through multiple mechanisms.

At least some of the T cell death seen in HIV$^+$ patients is believed to be mediated by TRAIL. While not wishing to be bound by theory, such TRAIL-mediated T cell death is believed to occur through the mechanism known as activation-induced cell death (AICD).

Activated human T cells are induced to undergo programmed cell death (apoptosis) upon triggering through the CD3/T cell receptor complex, a process termed activated-induced cell death (AICD). AICD of CD4$^+$ T cells isolated from HIV-infected aymptomatic individuals has been reported (Groux et al., *supra*). Thus, AICD may play a role in the depletion of CD4$^+$ T cells and the progression to AIDS in HIV-infected individuals.

The present invention provides a method of inhibiting TRAIL-mediated T cell death in HIV$^+$ patients, comprising administering TRAIL-BP (preferably, a soluble TRAIL-BP polypeptide) to the patients. In one embodiment, the patient is asymptomatic when treatment with TRAIL-BP commences. If desired, prior to treatment, peripheral blood T cells may be extracted from an HIV$^+$ patient, and tested for susceptibility to TRAIL-mediated cell death by conventional procedures.

In one embodiment, a patient's blood or plasma is contacted with TRAIL-BP *ex vivo*. The TRAIL-BP may be bound to a suitable chromatography matrix by conventional

procedures. The patient's blood or plasma flows through a chromatography column containing TRAIL-BP bound to the matrix, before being returned to the patient. The immobilized TRAIL-BP binds TRAIL, thus removing TRAIL protein from the patient's blood.

In treating HIV+ patients, a TRAIL-BP may be employed in combination with other inhibitors of T cell apoptosis. Fas-mediated apoptosis also has been implicated in loss of T cells in HIV+ individuals (Katsikis et al., *J. Exp. Med.* 181:2029-2036, 1995). Thus, a patient susceptible to both Fas ligand (Fas-L) mediated and TRAIL mediated T cell death may be treated with both an agent that blocks TRAIL/TRAIL-R interactions and an agent that blocks Fas-L/Fas interactions. Suitable agents for blocking binding of Fas-L to Fas include, but are not limited to, soluble Fas polypeptides; oligomeric forms of soluble Fas polypeptides (e.g., dimers of sFas/Fc); anti-Fas antibodies that bind Fas without transducing the biological signal that results in apoptosis; anti-Fas-L antibodies that block binding of Fas-L to Fas; and muteins of Fas-L that bind Fas but don't transduce the biological signal that results in apoptosis. Preferably, the antibodies employed in the method are monoclonal antibodies. Examples of suitable agents for blocking Fas-L/Fas interactions, including blocking anti-Fas monoclonal antibodies, are described in WO 95/10540, hereby incorporated by reference.

Compositions comprising an effective amount of a TRAIL-BP polypeptide of the present invention, in combination with other components such as a physiologically acceptable diluent, carrier, or excipient, are provided herein. TRAIL-BP can be formulated according to known methods used to prepare pharmaceutically useful compositions. TRAIL-BP can be combined in admixture, either as the sole active material or with other known active materials suitable for a given indication, with pharmaceutically acceptable diluents (e.g., saline, Tris-HCl, acetate, and phosphate buffered solutions), preservatives (e.g., thimerosal, benzyl alcohol, parabens), emulsifiers, solubilizers, adjuvants and/or carriers. Suitable formulations for pharmaceutical compositions include those described in *Remington's Pharmaceutical Sciences*, 16th ed. 1980, Mack Publishing Company, Easton, PA.

In addition, such compositions can contain TRAIL-BP complexed with polyethylene glycol (PEG), metal ions, or incorporated into polymeric compounds such as polyacetic acid, polyglycolic acid, hydrogels, dextran, etc., or incorporated into liposomes, microemulsions, micelles, unilamellar or multilamellar vesicles, erythrocyte ghosts or spheroblasts. Such compositions will influence the physical state, solubility, stability, rate of *in vivo* release, and rate of *in vivo* clearance of TRAIL-BP, and are thus chosen according to the intended application. TRAIL-BP expressed on the surface of a cell may find use, as well.

Compositions of the present invention may contain a TRAIL-BP polypeptide in any form described herein, such as native proteins, variants, derivatives, oligomers, and biologically active fragments. In particular embodiments, the composition comprises a soluble TRAIL-BP polypeptide or an oligomer comprising soluble TRAIL-BP polypeptides. The additional blocking agents described above may be included in the TRAIL-BP composition, or may be formulated separately.

TRAIL-BP can be administered in any suitable manner, e.g., topically, parenterally, or by inhalation. The term "parenteral" includes injection, e.g., by subcutaneous, intravenous, or intramuscular routes, also including localized administration, e.g., at a site of disease or injury. Sustained release from implants is also contemplated. One skilled in the pertinent art will recognize that suitable dosages will vary, depending upon such factors as the nature of the disorder to be treated, the patient's body weight, age, and general condition, and the route of administration. Preliminary doses can be determined according to animal tests, and the scaling of dosages for human administration are performed according to art-accepted practices.

Compositions comprising TRAIL-BP nucleic acids in physiologically acceptable formulations are also contemplated. TRAIL-BP DNA may be formulated for injection, for example.

Antibodies

Antibodies that are immunoreactive with TRAIL-BP polypeptides are provided herein. Such antibodies specifically bind TRAIL-BP, in that the antibodies bind to TRAIL-BP *via* the antigen-binding sites of the antibody (as opposed to non-specific binding).

The TRAIL-BP protein of Figure 1 (SEQ ID NO:2) may be employed as an immunogen in producing antibodies immunoreactive therewith. Alternatively, another form of TRAIL-BP, such as a fragment or fusion protein, may be employed as the immunogen. The present invention thus provides antibodies obtained by immunizing an animal with the TRAIL-BP of Figure 1, or an immunogenic fragment thereof. A method for producing antibodies comprises immunizing an animal with a TRAIL-BP polypeptide, whereby antibodies directed against the TRAIL-BP are generated in said animal. The desired antibodies may be purified, e.g., from the animal's serum, by conventional techniques.

Among the procedures for preparing polyclonal and monoclonal antibodies are those described in *Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses*, Kennet et al. (eds.), Plenum Press, New York (1980); and *Antibodies: A Laboratory Manual* , Harlow and Land (eds.), Cold Spring Harbor Laboratory Press, Cold

Spring Harbor, NY, (1988). Production of monoclonal antibodies directed against TRAIL-BP is further illustrated in example 4.

Antigen-binding fragments of antibodies directed against TRAIL-BP may be produced by well known procedures, and are encompassed by the present invention. Examples of such fragments include, but are not limited to, Fab and $F(ab')_2$ fragments. Antibody fragments and derivatives produced by genetic engineering techniques are also provided.

The monoclonal antibodies of the present invention include chimeric antibodies, e.g., humanized versions of murine monoclonal antibodies. Such humanized antibodies may be prepared by known techniques, and offer the advantage of reduced immunogenicity when the antibodies are administered to humans. In one embodiment, a humanized monoclonal antibody comprises the variable region of a murine antibody (or just the antigen binding site thereof) and a constant region derived from a human antibody. Alternatively, a humanized antibody fragment may comprise the antigen binding site of a murine monoclonal antibody and a variable region fragment (lacking the antigen-binding site) derived from a human antibody. Procedures for the production of chimeric and further engineered monoclonal antibodies include those described in Riechmann et al. (*Nature 332*:323, 1988), Liu et al. (*PNAS 84*:3439, 1987), Larrick et al. (*Bio/Technology 7*:934, 1989), and Winter and Harris (*TIPS 14*:139, May, 1993).

In one embodiment, the antibodies are specific for TRAIL-BP, and do not cross-react with other (non-TRAIL-BP) proteins. Screening procedures by which such antibodies may be identified are well known, and may involve immunoaffinity chromatography, for example.

Hybridoma cell lines that produce monoclonal antibodies specific for TRAIL-BP are also contemplated herein. Such hybridomas may be produced and identified by conventional techniques. One method for producing such a hybridoma cell line comprises immunizing an animal with a TRAIL-BP; harvesting spleen cells from the immunized animal; fusing said spleen cells to a myeloma cell line, thereby generating hybridoma cells; and identifying a hybridoma cell line that produces a monoclonal antibody that binds TRAIL-BP. The monoclonal antibodies may be recovered by conventional techniques.

Among the uses of the antibodies is use in assays to detect the presence of TRAIL-BP polypeptides, either *in vitro* or *in vivo*. The antibodies also may be employed in purifying TRAIL-BP proteins by immunoaffinity chromatography.

In one embodiment, the antibodies additionally can block binding of TRAIL to TRAIL-BP. Such antibodies may be employed to inhibit binding of TRAIL to cell surface TRAIL-BP, for example. Blocking antibodies may be identified using conventional assay procedures.

Such an antibody may be employed in an *in vitro* procedure, or administered *in vivo* to inhibit a TRAIL-BP-mediated biological activity. Disorders caused or exacerbated (directly or indirectly) by the interaction of TRAIL with cell surface TRAIL-BP thus may be treated. A therapeutic method involves *in vivo* administration of a blocking antibody to a mammal in an amount effective in inhibiting a TRAIL-mediated biological activity. Disorders caused or exacerbated, directly or indirectly, by the interaction of TRAIL with TRAIL-BP are thus treated. Monoclonal antibodies are generally preferred for use in such therapeutic methods. In one embodiment, an antigen-binding antibody fragment is employed.

Antibodies raised against TRAIL-BP may be screened for agonistic (i.e., ligand-mimicking) properties. Such antibodies may be tested for the ability to induce particular biological effects, upon binding to cell surface TRAIL-BP. Agonistic antibodies may be screened for activities reported for TRAIL, such as the ability to induce apoptosis of certain cancer cells (e.g., leukemia, lymphoma, and melanoma cells) or virally infected cells. (See Wiley et al., *Immunity* 3:673-682, 1995; and PCT application WO 97/01633.)

Compositions comprising an antibody that is directed against TRAIL-BP, and a physiologically acceptable diluent, excipient, or carrier, are provided herein. Suitable components of such compositions are as described above for compositions containing TRAIL-BP proteins.

Also provided herein are conjugates comprising a detectable (e.g., diagnostic) or therapeutic agent, attached to an antibody directed against TRAIL-BP. Examples of such agents are presented above. The conjugates find use in *in vitro* or *in vivo* procedures.

Nucleic Acids

The present invention provides TRAIL-BP nucleic acids. Examples of such nucleic acids include, but are not limited to, isolated DNAs comprising the nucleotide sequence presented in Figure 1 (SEQ ID NO:1), the coding region thereof, or fragments thereof.

The present invention provides isolated nucleic acids useful in the production of TRAIL-BP polypeptides, e.g., in the recombinant expression systems discussed above. Such nucleic acids include, but are not limited to, the human TRAIL-BP DNA of Figure 1 (SEQ ID NO:1). Nucleic acid molecules of the present invention include TRAIL-BP DNA in both single-stranded and double-stranded form, as well as the RNA complement thereof. TRAIL-BP DNA includes, for example, cDNA, genomic DNA, chemically synthesized DNA, DNA amplified by PCR, and combinations thereof. Genomic DNA may be isolated by conventional techniques, e.g., using the DNA of Figure 1 (SEQ ID NO:1), or a suitable fragment thereof, as a probe.

DNAs encoding TRAIL-BP in any of the forms contemplated herein (e.g., full length TRAIL-BP or fragments thereof) are provided. Particular embodiments of TRAIL-BP-encoding DNAs include a DNA encoding the full length human TRAIL-BP of Figure 1 and SEQ ID NO:2 (including the N-terminal signal peptide), and a DNA encoding a full length mature human TRAIL-BP. Other embodiments include DNA encoding a soluble TRAIL-BP (e.g., encoding the extracellular domain of the protein of Figure 1 and SEQ ID NO:2, either with or without the signal peptide).

Particular embodiments include TRAIL-BP-encoding DNAs comprising either of the above-discussed initiation codons. Thus, examples of such DNAs include those in which the coding region begins with the initiation codon (ATG) presented as nucleotides 24-26 of Figure 1 (SEQ ID NO:1); alternatively, the DNA may be truncated at the 5' end, such that the coding region begins with the ATG presented as nucleotides 144-146 of Figure 1 (SEQ ID NO:1).

Fragments of TRAIL-BP nucleotide sequences comprising at least about 17 nucleotides find use as probes or primers, for example. Such oligonucleotides may be employed as primers in polymerase chain reactions (PCR), whereby TRAIL-BP DNA fragments are isolated and amplified. Alternatively, a DNA fragment may comprise at least 30, or at least 60, contiguous nucleotides of a TRAIL-BP DNA sequence. To illustrate, a probe derived from a fragment of the DNA of Figure 1 (SEQ ID NO:1) may be used to screen a suitable cDNA library to identify TRAIL-BP clones. Examples of human cDNA libraries that may be employed include libraries derived from fetal liver and spleen, pregnant uterine tissue, foreskin fibroblasts, and peripheral blood leukocytes.

Other useful fragments of the TRAIL-BP nucleic acids include antisense or sense oligonucleotides comprising a single-stranded nucleic acid sequence (either RNA or DNA) capable of binding to target TRAIL-BP mRNA (sense) or TRAIL-BP DNA (antisense) sequences. Antisense or sense oligonucleotides, according to the present invention, comprise a fragment of the coding region of TRAIL-BP DNA. Such a fragment generally comprises at least about 14 nucleotides, preferably from about 14 to about 30 nucleotides. The ability to derive an antisense or a sense oligonucleotide, based upon a cDNA sequence encoding a given protein is described in, for example, Stein and Cohen (*Cancer Res.* 48:2659, 1988) and van der Krol et al. (*BioTechniques* 6:958, 1988).

Binding of antisense or sense oligonucleotides to target nucleic acid sequences results in the formation of duplexes that block transcription or translation of the target sequence by one of several means, including enhanced degradation of the duplexes, premature termination of transcription or translation, or by other means. The antisense oligonucleotides thus may be used to block expression of TRAIL-BP proteins. Antisense or sense oligonucleotides further comprise oligonucleotides having modified sugar-phosphodiester backbones (or other sugar linkages, such as those described in

WO91/06629) and wherein such sugar linkages are resistant to endogenous nucleases. Such oligonucleotides with resistant sugar linkages are stable *in vivo* (i.e., capable of resisting enzymatic degradation) but retain sequence specificity to be able to bind to target nucleotide sequences.

Other examples of sense or antisense oligonucleotides include those oligonucleotides which are covalently linked to organic moieties, such as those described in WO 90/10448, and other moieties that increases affinity of the oligonucleotide for a target nucleic acid sequence, such as poly-(L-lysine). Further still, intercalating agents, such as ellipticine, and alkylating agents or metal complexes may be attached to sense or antisense oligonucleotides to modify binding specificities of the antisense or sense oligonucleotide for the target nucleotide sequence.

Antisense or sense oligonucleotides may be introduced into a cell containing the target nucleic acid sequence by any gene transfer method, including, for example, $CaPO_4$-mediated DNA transfection, electroporation, or by using gene transfer vectors such as Epstein-Barr virus. In a preferred procedure, an antisense or sense oligonucleotide is inserted into a suitable retroviral vector. A cell containing the target nucleic acid sequence is contacted with the recombinant retroviral vector, either *in vivo* or *ex vivo*. Suitable retroviral vectors include, but are not limited to, those derived from the murine retrovirus M-MuLV, N2 (a retrovirus derived from M-MuLV), or the double copy vectors designated DCT5A, DCT5B and DCT5C (see WO 90/13641).

Sense or antisense oligonucleotides also may be introduced into a cell containing the target nucleotide sequence by formation of a conjugate with a ligand binding molecule, as described in WO 91/04753. Suitable ligand binding molecules include, but are not limited to, cell surface receptors, growth factors, other cytokines, or other ligands that bind to cell surface receptors. Preferably, conjugation of the ligand binding molecule does not substantially interfere with the ability of the ligand binding molecule to bind to its corresponding molecule or receptor, or block entry of the sense or antisense oligonucleotide or its conjugated version into the cell.

Alternatively, a sense or an antisense oligonucleotide may be introduced into a cell containing the target nucleic acid sequence by formation of an oligonucleotide-lipid complex, as described in WO 90/10448. The sense or antisense oligonucleotide-lipid complex is preferably dissociated within the cell by an endogenous lipase.

The following examples are provided to further illustrate particular embodiments of the invention, and are not to be construed as limiting the scope of the present invention.

EXAMPLE 1: Expression of TRAIL-BP and Binding Assay

A human TRAIL-BP was expressed by the following procedures. The expressed protein was tested for the ability to bind TRAIL.

DNA comprising nucleotides 144 to 923 of Figure 1 (SEQ ID NO:1) (encoding amino acids 41 to 299 of SEQ ID NO:2) was amplified by polymerase chain reaction (PCR). The coding region of this DNA begins with the second (downstream) initiation codon of the Figure 1 (SEQ ID NO:1) sequence, as discussed above. The isolated and amplified DNA was inserted into expression vector pDC409, to yield a construct designated pDC409-TRAIL-BP.

The expression vector designated pDC409 is a mammalian expression vector derived from the pDC406 vector described in McMahan et al. (*EMBO J.* 10:2821-2832, 1991; hereby incorporated by reference). Features added to pDC409 (compared to pDC406) include additional unique restriction sites in the multiple cloning site (mcs); three stop codons (one in each reading frame) positioned downstream of the mcs; and a T7 polymerase promoter, downstream of the mcs, that faciliates sequencing of DNA inserted into the mcs.

TRAIL-BP was tested for the ability to bind TRAIL, in a slide binding assay. Such assays are described in Gearing et al. (*EMBO J.* 8:3667, 1989); McMahan et al. (*EMBO J.* 10:2821, 1991) and Goodwin et al. (*Eur. J. Immunol.* 23:2631, 1993), hereby incorporated by reference.

The assay was conducted as follows. CV-1/EBNA cells were transfected with pDC409-TRAIL-BP. The transfected cells were cultured on glass slides in DMEM supplemented with 10% fetal bovine serum, penicillin, streptomycin, and glutamine. 48 hours after transfection, cells were incubated with the LZ-TRAIL fusion protein described below (1 μg/ml) in 1 ml of binding media (RPMI 1640 containing 25 mg/ml bovine serum albumin, 2 mg/ml sodium azide, 20mM Hepes (pH 7.2), and 50 mg/ml nonfat dry milk). After 30 minutes' incubation, the slides were washed once with binding media. A ^{125}I-labeled antibody specific for the leucine zipper (LZ) moiety of the fusion protein was then added (1 nM in 1 ml binding media). After 30 minutes' incubation, the slides were washed, fixed, and dipped in photographic emulsion.

The CV-1/EBNA cells transfected with the pDC409-TRAIL-BP expression vector showed significantly enhanced binding of LZ-TRAIL, compared to cells transfected with the empty pDC409 vector alone (a control).

The LZ-TRAIL protein employed in the foregoing binding assay is a fusion protein comprising a leucine zipper peptide fused to the N-terminus of a soluble TRAIL polypeptide. An expression construct was prepared, essentially as described for preparation of the Flag®-TRAIL expression construct in Wiley et al. (*Immunity*, 3:673-682, 1995; hereby incorporated by reference), except that DNA encoding the Flag® peptide was replaced with a sequence encoding a modified leucine zipper that allows for trimerization. The construct, in expression vector pDC409, encoded a leader sequence derived from human cytomegalovirus, followed by the leucine zipper moiety fused to the N-terminus of a soluble TRAIL polypeptide. The TRAIL polypeptide comprised amino acids 95-281 of human TRAIL (a fragment of the extracellular domain), as described in Wiley et al. (*supra*). The LZ-TRAIL was expressed in CHO cells, and purified from the culture supernatant.

EXAMPLE 2: Monoclonal Antibodies That Bind TRAIL-BP

This example illustrates a method for preparing monoclonal antibodies that bind TRAIL-BP. Suitable immunogens that may be employed in generating such antibodies include, but are not limited to, purified TRAIL-BP protein or an immunogenic fragment thereof such as the extracellular domain, or fusion proteins containing TRAIL-BP (e.g., a soluble TRAIL-BP/Fc fusion protein).

Purified TRAIL-BP can be used to generate monoclonal antibodies immunoreactive therewith, using conventional techniques such as those described in U.S. Patent 4,411,993. Briefly, mice are immunized with TRAIL-BP immunogen emulsified in complete Freund's adjuvant, and injected in amounts ranging from 10-100 μg subcutaneously or intraperitoneally. Ten to twelve days later, the immunized animals are boosted with additional TRAIL-BP emulsified in incomplete Freund's adjuvant. Mice are periodically boosted thereafter on a weekly to bi-weekly immunization schedule. Serum samples are periodically taken by retro-orbital bleeding or tail-tip excision to test for TRAIL-BP antibodies by dot blot assay, ELISA (Enzyme-Linked Immunosorbent Assay) or inhibition of TRAIL binding.

Following detection of an appropriate antibody titer, positive animals are provided one last intravenous injection of TRAIL-BP in saline. Three to four days later, the animals are sacrificed, spleen cells harvested, and spleen cells are fused to a murine myeloma cell line, e.g., NS1 or preferably P3x63Ag8.653 (ATCC CRL 1580). Fusions generate hybridoma cells, which are plated in multiple microtiter plates in a HAT

(hypoxanthine, aminopterin and thymidine) selective medium to inhibit proliferation of non-fused cells, myeloma hybrids, and spleen cell hybrids.

The hybridoma cells are screened by ELISA for reactivity against purified TRAIL-BP by adaptations of the techniques disclosed in Engvall et al., *Immunochem.* 8:871, 1971 and in U.S. Patent 4,703,004. A preferred screening technique is the antibody capture technique described in Beckmann et al., (*J. Immunol.* 144:4212, 1990) Positive hybridoma cells can be injected intraperitoneally into syngeneic BALB/c mice to produce ascites containing high concentrations of anti-TRAIL-BP monoclonal antibodies. Alternatively, hybridoma cells can be grown *in vitro* in flasks or roller bottles by various techniques. Monoclonal antibodies produced in mouse ascites can be purified by ammonium sulfate precipitation, followed by gel exclusion chromatography. Alternatively, affinity chromatography based upon binding of antibody to Protein A or Protein G can also be used, as can affinity chromatography based upon binding to TRAIL-BP.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: IMMUNEX CORPORATION

(ii) TITLE OF INVENTION: PROTEIN THAT BINDS TRAIL

(iii) NUMBER OF SEQUENCES: 3

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Kathryn A. Anderson, Immunex Corporation
(B) STREET: 51 University Street
(C) CITY: Seattle
(D) STATE: WA
(E) COUNTRY: :US
(F) ZIP: 98101

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: MS-DOS/Windows 95
(D) SOFTWARE: Word for Windows 95, 7.0a

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: --to be assigned--
(B) FILING DATE: 25-JUN-1998
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/883,529
(B) FILING DATE: 26-JUN-1997
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Anderson, Kathryn A.
(B) REGISTRATION NUMBER: 32,172
(C) REFERENCE/DOCKET NUMBER: 2629-WO

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 206-587-0430
(B) TELEFAX: 206-233-0644

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1347 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:
(B) CLONE: TRAIL-BP

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 24..920

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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GGCACGAGGG AGTTTGACCA GAG ATG CAA GGG GTG AAG GAG CGC TTC CTA          50
                          Met Gln Gly Val Lys Glu Arg Phe Leu
                            1               5

CCG TTA GGG AAC TCT GGG GAC AGA GCG CCC CGG CCG CCT GAT GGC CGA          98
Pro Leu Gly Asn Ser Gly Asp Arg Ala Pro Arg Pro Pro Asp Gly Arg
 10                  15                  20                  25

GGC AGG GTG CGA CCC AGG ACC CAG GAC GGC GTC GGG AAC CAT ACC ATG         146
Gly Arg Val Arg Pro Arg Thr Gln Asp Gly Val Gly Asn His Thr Met
                 30                  35                  40

GCC CGG ATC CCC AAG ACC CTA AAG TTC GTC GTC GTC ATC GTC GCG GTC         194
Ala Arg Ile Pro Lys Thr Leu Lys Phe Val Val Val Ile Val Ala Val
             45                  50                  55

CTG CTG CCA GTC CTA GCT TAC TCT GCC ACC ACT GCC CGG CAG GAG GAA         242
Leu Leu Pro Val Leu Ala Tyr Ser Ala Thr Thr Ala Arg Gln Glu Glu
         60                  65                  70

GTT CCC CAG CAG ACA GTG GCC CCA CAG CAA CAG AGG CAC AGC TTC AAG         290
Val Pro Gln Gln Thr Val Ala Pro Gln Gln Gln Arg His Ser Phe Lys
     75                  80                  85

GGG GAG GAG TGT CCA GCA GGA TCT CAT AGA TCA GAA CAT ACT GGA GCC         338
Gly Glu Glu Cys Pro Ala Gly Ser His Arg Ser Glu His Thr Gly Ala
 90                  95                 100                 105

TGT AAC CCG TGC ACA GAG GGT GTG GAT TAC ACC AAC GCT TCC AAC AAT         386
Cys Asn Pro Cys Thr Glu Gly Val Asp Tyr Thr Asn Ala Ser Asn Asn
                110                 115                 120

GAA CCT TCT TGC TTC CCA TGT ACA GTT TGT AAA TCA GAT CAA AAA CAT         434
Glu Pro Ser Cys Phe Pro Cys Thr Val Cys Lys Ser Asp Gln Lys His
            125                 130                 135

AAA AGT TCC TGC ACC ATG ACC AGA GAC ACA GTG TGT CAG TGT AAA GAA         482
Lys Ser Ser Cys Thr Met Thr Arg Asp Thr Val Cys Gln Cys Lys Glu
        140                 145                 150

GGC ACC TTC CGG AAT GAA AAC TCC CCA GAG ATG TGC CGG AAG TGT AGC         530
Gly Thr Phe Arg Asn Glu Asn Ser Pro Glu Met Cys Arg Lys Cys Ser
    155                 160                 165

AGG TGC CCT AGT GGG GAA GTC CAA GTC AGT AAT TGT ACG TCC TGG GAT         578
Arg Cys Pro Ser Gly Glu Val Gln Val Ser Asn Cys Thr Ser Trp Asp
170                 175                 180                 185

GAT ATC CAG TGT GTT GAA GAA TTT GGT GCC AAT GCC ACT GTG GAA ACC         626
Asp Ile Gln Cys Val Glu Glu Phe Gly Ala Asn Ala Thr Val Glu Thr
                190                 195                 200

CCA GCT GCT GAA GAG ACA ATG AAC ACC AGC CCG GGG ACT CCT GCC CCA         674
Pro Ala Ala Glu Glu Thr Met Asn Thr Ser Pro Gly Thr Pro Ala Pro
            205                 210                 215

GCT GCT GAA GAG ACA ATG AAC ACC AGC CCA GGG ACT CCT GCC CCA GCT         722
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Ala Ala Glu Glu Thr Met Asn Thr Ser Pro Gly Thr Pro Ala Pro Ala
       220                 225                 230

GCT GAA GAG ACA ATG ACC ACC AGC CCG GGG ACT CCT GCC CCA GCT GCT      770
Ala Glu Glu Thr Met Thr Thr Ser Pro Gly Thr Pro Ala Pro Ala Ala
    235                 240                 245

GAA GAG ACA ATG ACC ACC AGC CCG GGG ACT CCT GCC CCA GCT GCT GAA      818
Glu Glu Thr Met Thr Thr Ser Pro Gly Thr Pro Ala Pro Ala Ala Glu
250                 255                 260                 265

GAG ACA ATG ACC ACC AGC CCG GGG ACT CCT GCC TCT TCT CAT TAC CTC      866
Glu Thr Met Thr Thr Ser Pro Gly Thr Pro Ala Ser Ser His Tyr Leu
                270                 275                 280

TCA TGC ACC ATC GTA GGG ATC ATA GTT CTA ATT GTG CTT CTG ATT GTG      914
Ser Cys Thr Ile Val Gly Ile Ile Val Leu Ile Val Leu Leu Ile Val
            285                 290                 295

TTT GTT TGAAAGACTT CACTGTGGAA GAAATTCCTT CCTTACCTGA AAGGTTCAGG      970
Phe Val

TAGGCGCTGG CTGAGGGCGG GGGGCGCTGG ACACTCTCTG CCCTGCCTCC CTCTGCTGTG  1030

TTCCCACAGA CAGAAACGCC TGCCCCTGCC CCAAGTCCTG GTGTCTCCAG CCTGGCTCTA  1090

TCTTCCTCCT TGTGATCGTC CCATCCCCAC ATCCCGTGCA CCCCCCAGGA CCCTGGTCTC  1150

ATCAGTCCCT CTCCTGGAGC TGGGGGTCCA CACATCTCCC AGCCAAGTCC AAGAGGGCAG  1210

GGCCAGTTCC TCCCATCTTC AGGCCCAGCC AGGCAGGGGG CAGTCGGCTC CTCAACTGGG  1270

TGACAAGGGT GAGGATGAGA AGTGGTCACG GGATTTATTC AGCCTTGGTC AGAGCAGAAA  1330

AAAAAAAAAA AAAAAAA                                                 1347
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 299 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Gln Gly Val Lys Glu Arg Phe Leu Pro Leu Gly Asn Ser Gly Asp
  1               5                  10                  15

Arg Ala Pro Arg Pro Pro Asp Gly Arg Gly Arg Val Arg Pro Arg Thr
            20                  25                  30

Gln Asp Gly Val Gly Asn His Thr Met Ala Arg Ile Pro Lys Thr Leu
        35                  40                  45

Lys Phe Val Val Val Ile Val Ala Val Leu Leu Pro Val Leu Ala Tyr
    50                  55                  60

Ser Ala Thr Thr Ala Arg Gln Glu Glu Val Pro Gln Gln Thr Val Ala
65                  70                  75                  80
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Pro Gln Gln Gln Arg His Ser Phe Lys Gly Glu Glu Cys Pro Ala Gly
                 85                  90                  95

Ser His Arg Ser Glu His Thr Gly Ala Cys Asn Pro Cys Thr Glu Gly
            100                 105                 110

Val Asp Tyr Thr Asn Ala Ser Asn Asn Glu Pro Ser Cys Phe Pro Cys
        115                 120                 125

Thr Val Cys Lys Ser Asp Gln Lys His Lys Ser Ser Cys Thr Met Thr
    130                 135                 140

Arg Asp Thr Val Cys Gln Cys Lys Glu Gly Thr Phe Arg Asn Glu Asn
145                 150                 155                 160

Ser Pro Glu Met Cys Arg Lys Cys Ser Arg Cys Pro Ser Gly Glu Val
                165                 170                 175

Gln Val Ser Asn Cys Thr Ser Trp Asp Asp Ile Gln Cys Val Glu Glu
            180                 185                 190

Phe Gly Ala Asn Ala Thr Val Glu Thr Pro Ala Ala Glu Glu Thr Met
        195                 200                 205

Asn Thr Ser Pro Gly Thr Pro Ala Pro Ala Ala Glu Glu Thr Met Asn
    210                 215                 220

Thr Ser Pro Gly Thr Pro Ala Pro Ala Ala Glu Glu Thr Met Thr Thr
225                 230                 235                 240

Ser Pro Gly Thr Pro Ala Pro Ala Ala Glu Glu Thr Met Thr Thr Ser
                245                 250                 255

Pro Gly Thr Pro Ala Pro Ala Ala Glu Glu Thr Met Thr Thr Ser Pro
            260                 265                 270

Gly Thr Pro Ala Ser Ser His Tyr Leu Ser Cys Thr Ile Val Gly Ile
        275                 280                 285

Ile Val Leu Ile Val Leu Leu Ile Val Phe Val
    290                 295
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(vii) IMMEDIATE SOURCE:
(B) CLONE: FLAG peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Asp Tyr Lys Asp Asp Asp Asp Lys
1               5
```

What is claimed is:

1. An isolated DNA encoding a TRAIL-Binding Protein (TRAIL-BP), wherein said TRAIL-BP is selected from the group consisting of:

a) the TRAIL-BP polypeptide of SEQ ID NO:2; and

b) a fragment of the polypeptide of (a), wherein said fragment is capable of binding TRAIL.

2. A DNA of claim 1, wherein said TRAIL-BP is a soluble polypeptide comprising the extracellular domain of the TRAIL-BP of SEQ ID NO:2.

3. A DNA of claim 1, wherein said TRAIL-BP is a soluble fragment of the extracellular domain of the TRAIL-BP of SEQ ID NO:2.

4. A DNA of claim 1, wherein said DNA encodes an amino acid sequence selected from the group consisting of:

a) residues 1 to 299 of SEQ ID NO:2;

b) residues 41 to 299 of SEQ ID NO:2;

c) residues 1 to 278 of SEQ ID NO:2; and

d) residues 41 to 278 of SEQ ID NO:2.

5. A DNA of claim 1, wherein said TRAIL-BP comprises amino acids y to z of SEQ ID NO:2, wherein y represents an integer from 64 to 109, and z represents an integer from 189 to 299.

6. A DNA of claim 5, wherein y represents an integer from 64 through 70, and z is selected from the group consisting of 278 and 299.

7. A DNA of claim 5, wherein y is selected from the group consisting of 64, 66, 70, 108, or 109, and z is selected from the group consisting of 189, 190, 278, and 299.

8. An isolated DNA encoding a TRAIL-BP polypeptide, wherein said DNA encodes a polypeptide comprising an amino acid sequence that is at least 90% identical to the amino acid sequence presented in SEQ ID NO:2.

9. An isolated DNA encoding a soluble TRAIL-BP polypeptide, wherein said soluble TRAIL-BP comprises an amino acid sequence that is at least 90% identical to the sequence of residues 70 to 278 of SEQ ID NO:2.

10. A TRAIL-BP DNA of claim 8, wherein said TRAIL-BP polypeptide is naturally occurring.

11. An expression vector comprising a DNA according to claim 1.
12. An expression vector comprising a DNA according to claim 2.
13. An expression vector comprising a DNA according to claim 3.
14. An expression vector comprising a DNA according to claim 4.
15. An expression vector comprising a DNA according to claim 5.
16. An expression vector comprising a DNA according to claim 8.
17. An expression vector comprising a DNA according to claim 9.

18. A process for preparing a TRAIL-BP polypeptide, comprising culturing a host cell transformed with a vector according to claim 11 under conditions promoting expression of TRAIL-BP, and recovering the TRAIL-BP polypeptide.

19. A process for preparing a TRAIL-BP polypeptide, comprising culturing a host cell transformed with a vector according to claim 12 under conditions promoting expression of TRAIL-BP, and recovering the TRAIL-BP polypeptide.

20. A process for preparing a TRAIL-BP polypeptide, comprising culturing a host cell transformed with a vector according to claim 13 under conditions promoting expression of TRAIL-BP, and recovering the TRAIL-BP polypeptide.

21. A process for preparing a TRAIL-BP polypeptide, comprising culturing a host cell transformed with a vector according to claim 14 under conditions promoting expression of TRAIL-BP, and recovering the TRAIL-BP polypeptide.

22. A process for preparing a TRAIL-BP polypeptide, comprising culturing a host cell transformed with a vector according to claim 15 under conditions promoting expression of TRAIL-BP, and recovering the TRAIL-BP polypeptide.

23. A process for preparing a TRAIL-BP polypeptide, comprising culturing a host cell transformed with a vector according to claim 16 under conditions promoting expression of TRAIL-BP, and recovering the TRAIL-BP polypeptide.

24. A process for preparing a TRAIL-BP polypeptide, comprising culturing a host cell transformed with a vector according to claim 17 under conditions promoting expression of TRAIL-BP, and recovering the TRAIL-BP polypeptide.

25. A purified TRAIL-BP polypeptide selected from the group consisting of:

a) a mature form of the TRAIL-BP polypeptide of SEQ ID NO:2; and

b) a fragment of the polypeptide of (a), wherein said fragment is capable of binding TRAIL.

26. A TRAIL-BP of claim 25, wherein said TRAIL-BP is a soluble polypeptide comprising the extracellular domain of the TRAIL-BP of SEQ ID NO:2.

27. A TRAIL-BP of claim 25, wherein said TRAIL-BP is a soluble fragment of the extracellular domain of the TRAIL-BP of SEQ ID NO:2.

28. A TRAIL-BP of claim 25, wherein said TRAIL-BP comprises amino acids y to z of SEQ ID NO:2, wherein y represents an integer from 64 to 109, and z represents an integer from 189 to 299.

29. A TRAIL-BP of claim 28, wherein y represents an integer from 64 through 70, and z is selected from the group consisting of 278 and 299.

30. A TRAIL-BP of claim 28, wherein y is selected from the group consisting of 64, 66, 70, 108, or 109, and z is selected from the group consisting of 189, 190, 278, and 299.

31. A purified TRAIL-BP polypeptide comprising an amino acid sequence that is at least 90% identical to the amino acid sequence presented in SEQ ID NO:2.

32. A purified soluble TRAIL-BP polypeptide comprising an amino acid sequence that is at least 90% identical to the sequence of residues 70 to 278 of SEQ ID NO:2.

33. A TRAIL-BP of claim 31, wherein said TRAIL-BP polypeptide is naturally occurring.

34. An oligomer comprising from two to four TRAIL-BP polypeptides of claim 25.

35. An oligomer comprising from two to four TRAIL-BP polypeptides of claim 32.

36. A composition comprising a TRAIL-BP of claim 25, and a physiologically acceptable diluent, excipient, or carrier.

37. A composition comprising a TRAIL-BP of claim 32, and a physiologically acceptable diluent, excipient, or carrier.

38. A composition comprising an oligomer of claim 34, and a physiologically acceptable diluent, excipient, or carrier.

39. A composition comprising an oligomer of claim 35, and a physiologically acceptable diluent, excipient, or carrier.

40. An antibody that is directed against a TRAIL-BP polypeptide of claim 25, or an antigen-binding fragment of said antibody.

41. An antibody of claim 40, wherein the antibody is a monoclonal antibody.

42. A method of inhibiting a TRAIL-mediated activity, comprising contacting TRAIL with a TRAIL-BP of claim 25.

43. A method of inhibiting a TRAIL-mediated activity, comprising contacting TRAIL with a TRAIL-BP of claim 32.

44. A method of treating a TRAIL-mediated disorder, comprising administering a TRAIL-BP of claim 32 to a mammal afflicted with a TRAIL-mediated disorder.

45. A method of claim 44, wherein said TRAIL-mediated disorder is T cell death, and said mammal is an HIV^+ human.

FIGURE 1

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                                                                GGCACGAGGGAGTTTGACCAGAG   23

ATG CAA GGG GTG AAG GAG CGC TTC CTA CCG TTA GGG AAC TCT GGG GAC AGA GCG CCC CGG   83
Met Gln Gly Val Lys Glu Arg Phe Leu Pro Leu Gly Asn Ser Gly Asp Arg Ala Pro Arg   20

CCG CCT GAT GGC CGA GGC AGG GTG CGA CCC AGG ACC CAG GAC GGC GTC GGG AAC CAT ACC  143
Pro Pro Asp Gly Arg Gly Arg Val Arg Pro Arg Thr Gln Asp Gly Val Gly Asn His Thr   40

ATG GCC CGG ATC CCC AAG ACC CTA AAG TTC GTC GTC GTC ATC GTC GCG GTC CTG CTG CCA  203
Met Ala Arg Ile Pro Lys Thr Leu Lys Phe Val Val Val Ile Val Ala Val Leu Leu Pro   60

GTC CTA GCT TAC TCT GCC ACC ACT GCC CGG CAG GAG GAA GTT CCC CAG CAG ACA GTG GCC  263
Val Leu Ala Tyr Ser Ala Thr Thr Ala Arg Gln Glu Glu Val Pro Gln Gln Thr Val Ala   80

CCA CAG CAA CAG AGG CAC AGC TTC AAG GGG GAG GAG TGT CCA GCA GGA TCT CAT AGA TCA  323
Pro Gln Gln Gln Arg His Ser Phe Lys Gly Glu Glu Cys Pro Ala Gly Ser His Arg Ser  100

GAA CAT ACT GGA GCC TGT AAC CCG TGC ACA GAG GGT GTG GAT TAC ACC AAC GCT TCC AAC  383
Glu His Thr Gly Ala Cys Asn Pro Cys Thr Glu Gly Val Asp Tyr Thr Asn Ala Ser Asn  120

AAT GAA CCT TCT TGC TTC CCA TGT ACA GTT TGT AAA TCA GAT CAA AAA CAT AAA AGT TCC  443
Asn Glu Pro Ser Cys Phe Pro Cys Thr Val Cys Lys Ser Asp Gln Lys His Lys Ser Ser  140

TGC ACC ATG ACC AGA GAC ACA GTG TGT CAG TGT AAA GAA GGC ACC TTC CGG AAT GAA AAC  503
Cys Thr Met Thr Arg Asp Thr Val Cys Gln Cys Lys Glu Gly Thr Phe Arg Asn Glu Asn  160

TCC CCA GAG ATG TGC CGG AAG TGT AGC AGG TGC CCT AGT GGG GAA GTC CAA GTC AGT AAT  563
Ser Pro Glu Met Cys Arg Lys Cys Ser Arg Cys Pro Ser Gly Glu Val Gln Val Ser Asn  180

TGT ACG TCC TGG GAT GAT ATC CAG TGT GTT GAA GAA TTT GGT GCC AAT GCC ACT GTG GAA  623
Cys Thr Ser Trp Asp Asp Ile Gln Cys Val Glu Glu Phe Gly Ala Asn Ala Thr Val Glu  200

ACC CCA GCT GCT GAA GAG ACA ATG AAC ACC AGC CCG GGG ACT CCT GCC CCA GCT GCT GAA  683
Thr Pro Ala Ala Glu Glu Thr Met Asn Thr Ser Pro Gly Thr Pro Ala Pro Ala Ala Glu  220

GAG ACA ATG AAC ACC AGC CCA GGG ACT CCT GCC CCA GCT GCT GAA GAG ACA ATG ACC ACC  743
Glu Thr Met Asn Thr Ser Pro Gly Thr Pro Ala Pro Ala Ala Glu Glu Thr Met Thr Thr  240

AGC CCG GGG ACT CCT GCC CCA GCT GCT GAA GAG ACA ATG ACC ACC AGC CCG GGG ACT CCT  803
Ser Pro Gly Thr Pro Ala Pro Ala Ala Glu Glu Thr Met Thr Thr Ser Pro Gly Thr Pro  260

GCC CCA GCT GCT GAA GAG ACA ATG ACC ACC AGC CCG GGG ACT CCT GCC TCT TCT CAT TAC  863
Ala Pro Ala Ala Glu Glu Thr Met Thr Thr Ser Pro Gly Thr Pro Ala Ser Ser His Tyr  280

CTC TCA TGC ACC ATC GTA GGG ATC ATA GTT CTA ATT GTG CTT CTG ATT GTG TTT GTT TGA  923
Leu Ser Cys Thr Ile Val Gly Ile Ile Val Leu Ile Val Leu Leu Ile Val Phe Val End  299

AAGACTTCACTGTGGAAGAAATTCCTTCCTTACCTGAAAGGTTCAGGTAGGCGCTGGCTGAGGGCGGGGGGCGCTGGAC 1002
ACTCTCTGCCCTGCCTCCCTCTGCTGTGTTCCCACAGACAGAAACGCCTGCCCCTGCCCCAAGTCCTGGTGTCTCCAGC 1081
CTGGCTCTATCTTCCTCCTTGTGATCGTCCCATCCCCACATCCCGTGCACCCCCCAGGACCCTGGTCTCATCAGTCCCT 1160
CTCCTGGAGCTGGGGGTCCACACATCTCCCAGCCAAGTCCAAGAGGGCAGGGCCAGTTCCTCCCATCTTCAGGCCCAGC 1239
CAGGCAGGGGGCAGTCGGCTCCTCAACTGGGTGACAAGGGTGAGGATGAGAAGTGGTCACGGGATTTATTCAGCCTTGG 1318
TCAGAGCAGAAAAAAAAAAAAAAAAAAAA                                                    1347
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/13491

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07K 14/71, 16/28; C12N 5/10, 15/10, 15/12, 15/63; A61K 38/17
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.1, 253.4, 254.11, 325, 320.1; 530/350, 388.22; 536/23.1, 23.5; 514/12

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, EMBASE, WPIDS, CAPLUS
search terms: trial, protein?, polypeptide?, peptide?, bp, walczak h, smith craig a

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Database GenBank on STN. US National Library of Medicine (Bethesda, MD, USA). HILLIER et al. GenBank Accession Number AA150541. zl44b01.sl Soares pregnant uterus NbHPU Homo sapiens cDNA clone 504745 3'. 19 May 1997.	1-17
A	Database GenBank on STN. US National Library of Medicine (Bethesda, MD, USA). HILLIER et al. GenBank Accession Number AA150849. zl44b01.rl Soares pregnant uterus NbHPU Homo sapiens cDNA clone 504745 5'. 19 May 1997.	1-17
A	PAN et al. The receptor for the cytotoxic ligand TRAIL. Science. 04 April 1997, Vol. 276, pages 111-113, see entire document.	1-41

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 10 SEPTEMBER 1998	Date of mailing of the international search report 14 OCT 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer CLAIRE M. KAUFMAN Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/13491

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P ----- A,P	MARIAPIA et al. Cloning and characterization of TRAIL-R3, a novel member of the emerging TRAIL receptor family. J. Exp. Med. 06 October 1997, Vol. 186, No. 7, pages 1165-1170, see entire document.	1-33, 36, 37, 42, 43 ---------- 34, 35, 38-41, 44, 45

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/13491

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/13491

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/69.1, 253.4, 254.11, 325, 320.1; 530/350, 388.22; 536/23.1, 23.5; 514/12

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s)1-39, drawn to DNA,vector, and process of using DNA to produce a polypeptide, the polypeptide, oligomer, and composition comprising the polypeptide..
Group II, claim(s) 40-41, drawn to antibody.
Group III, claims 42-45, drawn to method of inhibiting and method of treating.
The inventions listed as Groups I-III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Pursuant to 37 CFR 1.475(d), this Authority considers that the main invention in the instant application comprises the first-recited product, the DNA, and the first recited method of using that product, namely a method of using the DNA to produce an encoded polypeptide; as well as the polypeptide, oligomer, and composition comprising the polypeptide. Further pursuant to 36 CFR 1.475(b)-(d), the ISA/US considers that the materially and functionally dissimilar product of Group II and the additional method of Group II does not correspond to the main invention. This Authority therefore considers that the several inventions do not share a special technical feature within the meaning of PCT Rule 13. 2 and thus do not relate to a single general inventive concept within the meaning of PCT Rule 13.1.